sequences encoding the molecule of interest may be desirable to achieve this end. For example, in some cases it may be necessary to modify the sequence so that it can be attached to the control sequences in the appropriate orientation; *i.e.*, to maintain the reading frame. The control sequences and other regulatory sequences may be ligated to the coding sequence prior to insertion into a vector. Alternatively, the coding sequence can be cloned directly into an expression vector which already contains the control sequences and an appropriate restriction site.

As explained above, it may also be desirable to produce mutants or analogs of the antigen of interest. Methods for doing so are described in, *e.g.*, Dasmahapatra *et al.*, US Pat. No. 5,843,752 and Zhang *et al.*, US Pat. No. 5,990,276. Mutants or analogs of SARSV proteins for use in the subject assays may be prepared by the deletion of a portion of the sequence encoding the polypeptide of interest, by insertion of a sequence, and/or by substitution of one or more nucleotides within the sequence. Techniques for modifying nucleotide sequences, such as site-directed mutagenesis, and the like, are well known to those skilled in the art. See, *e.g.*, Sambrook *et al.*, *supra*; Kunkel, T. A. (1985) *Proc. Natl. Acad. Sci. USA* (1985) 82:448; Geisselsoder *et al.* (1987) *BioTechniques* 5:786; Zoller & Smith (1983) *Methods Enzymol.* 100:468; Dalbie-McFarland *et al.* (1982) *Proc. Natl. Acad. Sci USA* 79:6409.

The molecules can be expressed in a wide variety of systems, including insect, mammalian, bacterial, viral and yeast expression systems, all well known in the art.

For example, insect cell expression systems, such as baculovirus systems, are known to those of skill in the art and described in, *e.g.*, Summers & Smith, *Texas Agricultural Experiment Station Bulletin* No. 1555 (1987). Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *inter alia*, Invitrogen, San Diego Calif. ("MaxBac" kit). Similarly, bacterial and mammalian cell expression systems are well known in the art and described in, *e.g.*, Sambrook *et al.*, *supra*. Yeast expression systems are also known in the art and described in, *e.g.*, *Yeast Genetic Engineering* (Barr *et al.*, eds., 1989) Butterworths, London.

A number of appropriate host cells for use with the above systems are also known. For example, mammalian cell lines are known in the art and include immortalized cell lines available from the American Type Culture Collection (ATCC), such as, but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human embryonic kidney cells, human hepatocellular carcinoma cells (*e.g.*, Hep G2), Madin-Darby bovine kidney ("MDBK") cells, as well as others. Similarly, bacterial hosts such as *E.coli*, *Bacillus subtilis*, and *Streptococcus* spp., will find use with the present expression constructs. Yeast hosts useful in the present invention include *inter alia*, *Saccharomyces cerevisiae, Candida albicans, Candida maltosa, Hansenula polymorpha, Kluyveromyces fragilis,*

Kluyveromyces lactis, Pichia guillerimondii, Pichia pastoris, Schizosaccharomyces pombe and *Yarrowia lipolytica.* Insect cells for use with baculovirus expression vectors include, *inter alia, Aedes aegypti, Autographa californica, Bombyx mori, Drosophila melanogaster, Spodoptera frugiperda, and Trichoplusia ni.*

Nucleic acid molecules comprising nucleotide sequences of interest can be stably integrated into a host cell genome or maintained on a stable episomal element in a suitable host cell using various gene delivery techniques well known in the art. See, *e.g.*, US Pat. No. 5,399,346.

Depending on the expression system and host selected, the molecules are produced by growing host cells transformed by an expression vector described above under conditions whereby the protein is expressed. The expressed protein is then isolated from the host cells and purified. If the expression system secretes the protein into growth media, the product can be purified directly from the media. If it is not secreted, it can be isolated from cell lysates. The selection of the appropriate growth conditions and recovery methods are within the skill of the art.

EXAMPLE

For useful expression of SARSV antigens in *Saccharomyces cerevisiae* and *Pichia pastoris*, insect cells, and mammalian cells, the following domains are cloned into expression vectors as listed in the Table below. The nt sequence numbers are from the SARSV sequence of SEQ ID NO: 1.

- RNA polymerase 1a: SARS nt 250-13398
- RNA polymerase 1b: SARS nt 13399-21470
- ORFns.envelope (homologous to ns2, hemagglutinin-esterase envelope glycoprotein, and spike glycoprotein): SARS nt 21477-25244
- Membrane: SARS nt 27849 - 28103
- Nucleocapsid: SARS nt 28105 - 29373

A combination of PCR and synthetic oligos is used to create the above domains with restriction sites tailored to the following expression vectors:

Restriction ends	**Vector**	**Promoter**	**Expression host**
*Hin*dIII/*Sal*I	pBS24.1	ADH2/GAPDH	AD3/Saccharomyces
*Eco*RI/*Sal*\\\	pBS24.1	ADH2/GAPDH/SOD fusion	AD3/Saccharomyces
*Xba*I/*Sal*I	pAO815	AOXI	GS115/Pichia pastoris
*Eco*RI/*Bam*HI	pCMVkm2	CMVp/Enhancer/IntronA	HVK-293/Transient transfection
*Eco*RI/*Xma*I	pCMVIII	CMVp/Enhancer/IntronA	CHO stable cell line
*Nhe*I/*Sal*I	pBluBac4.5	Polyhedrin	Cell lines employed by Chiron include: Sf9, Sf21, Tn5

IV. TREATMENT OF SARS INFECTION WITH RNAi

RNA interference or "RNAi" is a term initially coined by Fire and co-workers to describe the observation that double-stranded RNA (dsRNA) can block gene expression when it is introduced into worms (Fire *et al.*, Nature 391, 806-811(1998)). RNAi most likely involves mRNA degradation, resulting in sequence-specific, post-transcriptional gene silencing in many organisms. RNAi is a post-transcriptional process triggered by the introduction of double-stranded RNA which leads to gene silencing in a sequence-specific manner. RNAi has been reported to occur naturally in organisms as diverse as nematodes, trypanosmes, plants and fungi. It most likely serves to protect organisms from viruses, modulate transposon activity and eliminate aberrant transcription products.

The first evidence that dsRNA could achieve efficient gene silencing through RNAi came from studies on the nematode *Caenorhabditis elegans* (Fire *et al.* (1998) *Nature*, 391:806-811 and US Patent No. 6,506,559). Later studies in the fruit fly *Drosophila melanogaster* demonstrated that RNAi is a two-step mechanism (Elbashir *et al.* (2001) *Genes Dev.*, 15(2): 188-200). First, long dsRNAs are cleaved by an enzyme known as Dicer in 21-23 nucleotides (nt) fragments, called small interfering RNAs (siRNAs). Then, siRNAs associate with a ribonuclease complex (termed RISC for RNA Induced Silencing Complex) which target this complex to complementary mRNAs. RISC then cleaves the targeted mRNAs opposite the complementary siRNA, which makes the mRNA susceptible to other RNA degradation pathways.

RNAi is the phenomenon where dsRNA corresponding to a targeted DNA or RNA sequence can suppress or silence gene expression. Even though dsRNA can mediate gene-specific interference in mammalian cells in some circumstances (Wianny & Zernicka-Goetz (2000) *Nature Cell Biol.* 2:70-75; Svoboda *et al.* (2000) *Development* 17:4147-4156) the use of RNAi in mammalian somatic cells is often limited due to the dsRNA triggering dsRNA-dependent protein kinase (PKR) which in turn inactivates translation factor eIF2a and causes a generalized suppression of protein synthesis and often times apoptosis (Gil & Esteban (2000) *Apoptosis* 5:107-114).

Recently, gene-specific suppression using siRNA of approximately 21 or 22 base pairs in length, corresponding to targeted RNA or DNA sequences, were shown to disrupt the expression of these targeted sequences in mammalian cells (Elbashir, S.M., *et al.*, *Nature* 411: 494-498 (2001)). However, it is not clear that all RNA or DNA sequences of a mammalian cell's genome are susceptible to siRNA. It is also uncertain that every mammalian cell type possesses the necessary machinery for effecting gene-specific suppression using siRNA. Further, siRNA is of limited use for at least two reasons: the transient nature of the suppression effect seen in cells where the siRNA has been administered; and in some instances the necessity for chemical synthesis of siRNAs before their use (Tuschl T., *Nature Biotechnol.*, 20: 446-448 (2002)). Also

the instability of these short, synthetic RNAs makes it presents problems for any long term use of these siRNAs a pharmaceutical.

To overcome this limitation, the present invention provides a modified siRNA with increased stability against nuclease degradation while still maintaining its ability to inhibit viral replication via RNA interference. Such modification to the ribonucleotides in the siRNAs, adds a chemical group via chemical synthesis or *in vitro* transcription or longer modified RNAs can be prepared by either of these methods and cut into siRNAs using Dicer.

Although other methods for gene-specific suppression have utilized chemically-modified nucleic acids, such as antisense and ribozyme technology, such modification destroys critical enzymatic activities necessary for the function of these technologies. In regard to antisense technology, modification of the ribonucleotides destroys RNaseH activity, whereas such modification abolishes the catalytic activity of ribozymes.

The present invention provides a double-stranded RNA (dsRNA) molecule modified for protection against nuclease degradation with a length from about 10 to about 30 nucleotides which is able to inactivate a virus in a mammalian cell. The invention also provides a method of inactivating a virus by administering modified small interfering RNAs (siRNAs) that are modified so that they are nuclease or RNase resistant and retain the biological activity of being able to inhibit viral replication by targeting a RNA sequence in a virus.

The invention is further directed to a method of making modified siRNAs that target a RNA sequence in a virus comprising preparing a modified-double stranded RNA (dsRNA) fragment containing at least one modified ribonucleotide in at least one strand that spans the genome of the virus; and cleaving the modified-dsRNA fragments with recombinant human Dicer resulting in more than one modified siRNA.

The present invention provides a modified dsRNA molecule of from about 10 to about 30 nucleotides which mediates targeted RNA interference in hepatic or SARS-infected cells.

As used herein RNA interference, or RNAi, is used to mean sequence-specific, or gene specific, suppression of gene expression (protein synthesis), without causing a generalized suppression of protein synthesis in cells harboring the siRNA. The invention is not limited to a particular theory of the mechanism of action of RNAi. For example, RNAi may involve degradation of messenger RNA (mRNA) in an RNA-induced silencing complex (RISC), preventing translation of the transcribed mRNA, or it may involve the methylation of genomic DNA, shunting transcription of the gene. The lack of gene expression caused by RNAi may be transient, lasting a short period of time, or it may be stable, or permanent, lasting an indefinite period of time.

The term RNA is meant as is recognized in the art. Further, as used herein, RNA is used to mean double-stranded RNA (dsRNA) or single-stranded RNA (ssRNA) or a dsRNA with a

single-stranded overhang. dsRNAs within the meaning of the present invention includes short interfering RNA (siRNA), micro RNA (miRNA) and small hairpin RNA (shRNA), Additionally, RNA is also used to mean messenger RNA (mRNA), transfer RNA (tRNA) or ribosomal RNA (rRNA).

The present invention is directed to small interfering RNA (siRNA) which have been chemically modified to confer increased stability against nuclease degradation yet these siRNAs are still able to bind to target RNAs, that may be present in a cells. In the case where the target RNA is a virus specific RNA, the modified siRNAs are able to bind to the virus specific RNAs and inactivate the virus. A modified siRNA of the present invention comprises a modified ribonucleotide, wherein the siRNA is resistant to enzymatic degradation, such as RNase degradation, and yet retains the ability to inhibit viral replication. The modified siRNA is more specifically modified at the 2' position of the ribose in the siRNA. The modification is at the 2' position of at least one ribonucleotide of said siRNA. Attachment of receptor-binding ligands to siRNA molecules can be used to target the siRNA to a desired cell type. For example, attachment of cholesterol at the 5'-end or 3'-end of the siRNA molecule, to give a cholesteryl siRNA, can enhance targeting to hepatocytes. Other ligands for receptor mediated siRNA targeting to liver include HBV surface antigen, LDL, and others.

More specifically, the siRNA is modified at at least one pyrimidine, at least one purine or a combination thereof. However, generally all pyrimidines, or all purines or a combination of all pyrimidines and all purines of the siRNA are modified. More preferably, the pyrimidines are modified and these pyrimidines are cytosine, a derivative of cytosine, uracil, a derivative of uracil or a combination thereof. It also is contemplated to modify the selected ribonucleotides in at least one strand of the siRNA or the ribonucleotides in both strands of the siRNA are modified.

The nucleotides containing pyrimidine bases found in RNA (cytidine and uridine) can be chemically modified by adding any molecule that inhibits RNA degradation or breakdown to the 2' position of the ribose molecule. The 2'-modified pyrimidine nucleotide can be formed using a number of different methods. The 2' modification confers increased stability to the siRNA by making the siRNA impervious or resistant to nuclease activity. Thus, the 2' modified siRNA has a longer serum half-life and is resistant to degradation compared to unmodified siRNA. The siRNA also may be modified completely or partially.

Regarding chemical modification of siRNAs, a molecule from the halide chemical group is preferably added to the ribonucleotide of the siRNA. Within the halides, fluorine is the preferred molecule but other chemical molecules, in addition to fluoro-, such as methyl-, methoxyethyl- and propyl-modifications can also we made. But the preferred modications is fluoro-modification, such as a 2'-fluoro-modication or a 2',2'-fluoro-modification.Thus, in a preferred

embodiment of the invention, the siRNA is modified by adding a fluorine molecule to the 2' carbon of the pyrimidine ribonucleotide. The siRNA may be fluorinated completely or partially. For example, only the cytosine nucleotides need be fluorinated. Alternatively, only the uracil nucleotide need be fluorinated but both uracil and cytosine can be fluorinated. Furthermore, only one strand, either sense or antisense, of the siRNA can be fluorinated. Even partial 2' fluorination the siRNA gives protection against nucleolytic degradation. Furthermore, it is important to note the 2' fluorinated siRNA is not toxic to cells, an unexpected result given that fluorine chemistry usually is toxic to living organisms.

The siRNA of the present invention is designed to interact with a target nucleotide sequence. Most preferably this target nucleotide sequence is a disease producing agent or pathogen of which one wishes to inhibit gene expression. More preferably, this target nucleotide sequence is in a virus genome, and further this virus genome is from a RNA virus or a DNA virus is selected from the group consisting of hepatitis C virus (HCV), hepatitis A virus, hepatitis B virus, hepatitis D virus, hepatitis E virus, Ebola virus, influenza virus, rotavirus, reovirus, retrovirus, poliovirus, human papilloma virus (HPV), metapneumovirus and coronavirus. The most preferred virus is SARS virus.

Modfied siRNA may be prepared in a number of ways, such as by chemical synthesis, T7 polymerase transcription, or by treating modified long double stranded RNA (dsRNA) prepared by one of the two previous methods with Dicer enzyme. Dicer enzyme can be used to cleave dsRNA that is about 500 base pairs to about 1000 base pairs in size, to created mixed populations of dsRNA from about 21 to about 23 base pairs in length. Furthermore, an unexpected result of using the Dicer enzyme method is that Dicer enzyme will cleave modified strands of dsRNA, such as 2' fluorinated modified dsRNA. Before development of this method, it was previously thought that Dicer would not be able to cleave modified siRNA. The Dicer method can be carried out using the Dicer siRNA Generation Kit available from Gene Therapy Systems, San Diego, CA.

As used herein, small interfering RNA (siRNA) is defined as double- or single-stranded RNA of from about 10 to about 30 nucleotides in length, more preferably 12-28 nucleotides, more preferably 15-25 nucleotides, even more preferably 19-23 nucleotides and most preferably 21-23 nucleotides. The length of a siRNA as used herein, is determined by the length of one of the strands of the RNA. For example, a siRNA that is described as 21 nucleotides long (a 21-mer) may comprise two opposite strands of RNA which anneal together for 19 contiguous base pairings. The two remaining nucleotides on one end of the molecule would not anneal to the opposite strand, thus creating an "overhang". The overhang can be at the 5' or the 3' end of the dsRNA. Preferably, the overhang is at the 3' end of the RNA strand. The length of a double-stranded RNA where the two opposite strands are not the same length will be designated by the

longer of the two strands. For example, a dsRNA comprising one strand which is 21 nucleotides long and anneals to an opposite strand that is 20 nucleotides long, will be considered, as used herein, a 21-mer.

Preferably, the siRNA of the present invention will comprise a 3' overhang of about 2 to 4 bases. More preferably, the 3' overhang is 2 nucleotides long. Even more preferably, the 2 nucleotides comprising the 3' overhang are uridine (U).

In one embodiment, the invention provides an RNA molecule comprising a nucleotide sequence at least 80% identical to the nucleotide sequence of the target agent or virus. Preferably, the RNA molecule of the present invention is at least 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of the target agent or virus.

As a practical matter, whether any particular nucleic acid molecule is at least 90%, 95%, 96%, 97% 98%, 99% or 100% identical to the nucleotide sequence of the target agent or virus can be determined conventionally using known computer programs such as the *Bestfit* program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, Wis. 53711). *Bestfit* uses the local homology algorithm of Smith & Waterman (*Advances in Applied Mathematics* 2:482-489 (1981)) to find the best segment of homology between two sequences. When using *Bestfit* or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

The present invention provides a method of inactivating a target agent or preferably a virus in a patient comprising administering to the patient a modified siRNA in an effective amount to inactivate the targeted agent or virus. RNA interference towards a targeted DNA segment in a cell can be achieved by administering a dsRNA molecule or siRNA to the cells, wherein the nucleotide sequence of the dsRNA molecule corresponds to the nucleotide sequence of the targeted DNA segment. Preferably, the RNA molecule used to induce targeted RNAi is siRNA.

Gene suppression, targeted suppression, sequence-specific suppression, targeted RNAi or sequence-specific RNAi are used interchangeably herein. Furthermore, sequence-specific suppression, as used herein, is determined by separately assaying the levels of the protein targeted for suppression in cells containing the siRNA (experimental cells) and in cells not containing the identical siRNA (control cells), and comparing the two values. Furthermore, the experimental and control cells must be derived from the same source and same animal. For example, the control and experimental cells can be, but are not limited to, normal human hepatic cells as cell culture *in vitro*, or they can derived from a hepatocellular carcinoma. Further, the

control and experimental cells used in determining the level or quantity of gene suppression must be assayed under similar, if not identical, conditions.

As used herein the phrase "targeted DNA segment" is used to mean a DNA sequence encoding, in whole or in part, an mRNA for a targeted protein, including introns or exons, where suppression is desired. DNA segment can also mean a DNA sequence that normally regulates expression of the targeted protein, including but not limited to the promoter of the targeted protein. Furthermore, the DNA segment may or may not be a part of the cell's genome or it may be extrachromosomal, such as plasmid DNA.

The present invention is further directed to inactivating a virus in a patient comprising administering to a patient a modified siRNA in an effective amount to inactivate the virus. The siRNA is preferably about 10 to about 30 nucleotides in length, more preferably 12-28 nucleotides, more preferably 15-25 nucleotides, even more preferably 19-23 nucleotides and most preferably 21-23 nucleotides. The method preferably utilizes a 2' modified siRNA that is modified at the 2' position of at least one ribonucleotide of said siRNA. The method utilizes a siRNA that is modified with chemical groups selected from the group consisting of fluoro-, methyl-, methoxyethyl- and propyl-modification. The fluoro-modification is preferred and either a 2'-fluoro-modication or a 2',2'-fluoro-modification is useful in the present invention and preferred.

The modification may be at the pyrimidines, the purines or a combination thereof of the siRNA are modified. More preferably the pyrimidines are modified, such as cytosine, a derivative of cytosine, uracil, a derivative of uracil or a combination thereof. In one embodiment, at least one strand of the siRNA contains at least one modified nucleotide and in an alternate embodiment, oth strands of the siRNA contains at least one modified nucleotide.

The method is intended to target disease causing agents or pathogens, an more particularly viruses, which can be either a RNA virus or a DNA virus, which are selected from the group consisting of hepatitis C virus (HCV), hepatitis A virus, hepatitis B virus, hepatitis D virus, hepatitis E virus, Ebola virus, influenza virus, rotavirus, reovirus, retrovirus, poliovirus, human papilloma virus (HPV), metapneumovirus and coronavirus. More preferably the target virus is a SARS virus. The present method utilizes a siRNA prepared by (a) identifying a target nucleotide sequence in a virus genome, preferably SARS virus, for designing a small interfering RNA (siRNA); and (b) producing a siRNA that has been modified to contain at least one modified nucleotide. More preferably, the siRNA comprises a dsRNA molecule with a first strand ribonucleotide sequence corresponding to a nucleotide sequence corresponding to a target nucleotide sequence in said virus and a second strand comprising a ribonucleotide sequence complementary to said target nucleotide sequence, wherein said first and second strands are separate complementary strands that hybridize to each other to form said dsRNA molecule, and

further wherein the first strand ribonucleotide sequence, the second strand ribonucleotide sequence or both the first and second strand ribonucletide sequences comprise at least one modified nucleotide. In this method, the target nucleotide sequence comprises a conserved nucleotide sequence necessary for SARS virus replication, and the conserved nucleotide sequence is selected from the group consisting of SEQ ID NO: 7292, SEQ ID NO: 7293, SEQ ID NO: 7294, SEQ ID NO: 7295, SEQ ID NO: 7296, SEQ ID NO: 7297, SEQ ID NO: 7298, SEQ ID NO: 7299, SEQ ID NO: 7300 and SEQ ID NO: 7301. Preferably, the nucleotide sequence is selected from the group consisting of SEQ ID NO: 7292 and SEQ ID NO: 7293. Still more preferably, the nucleotide sequence is SEQ ID NO: 7293.

The siRNA disclosed in this application may be prepared with modified ribonucleotides as described herein. Further, the modified ribonucleotide of the siRNA used in the present method is incorporated into said siRNA by chemical synthesis or enzymatic synthesis.

The siRNA disclosed in this application may or may not have a 5' triphosphate group.

The modified siRNA is administered to a patient by a method selected from the group consisting of intravenous injection, subcutaneous injection, oral delivery, and liposome delivery. The modified siRNA accumulates in an organ, tissue or body system of the patient that are the liver, gastrointestinal tract, respiratory tract, cervix or skin.

The present invention also provides a method of inhibiting the replication of a virus, such as SARS virus, in cells positive for SARS virus comprising transfecting SARS-positive cells with a vector that directs the expression of modified siRNA which is specific for SARS. The cells are evaluated to determine if a marker in the cells has been inhibited by the modified siRNA.

The term patient, as used herein, can be an animal, preferably a mammal. More preferably the subject can be a primate, including non-human and humans. The terms subject and patient can be used interchangeably.

The treatment envisioned by the current invention can be used for subjects with a pre-existing viral infection, or for subjects pre-disposed to a SARS virus infection. Additionally, the method of the current invention can be used to correct or compensate for cellular or physiological abnormalities involved in conferring susceptibility to viral infections in patients, and/or to alleviate symptoms of a viral infection in patients, or as a preventative measure in patients.

The method of treating a patient having a viral infection involves administration of compositions to the subjects. As used herein, composition can mean a pure compound, agent or substance or a mixture of two or more compounds, agents or substances. As used herein, the term agent, substance or compound is intended to mean a protein, nucleic acid, carbohydrate, lipid, polymer or a small molecule, such as a drug.

In one embodiment of the current invention, the composition administered to the subject is a pharmaceutical composition. Further, the pharmaceutical composition can be administered orally, nasally, parenterally, intrasystemically, intraperitoneally, topically (as by drops or transdermal patch), bucally, or as an oral or nasal spray. The term "parenteral," as used herein, refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion. The pharmaceutical compositions as contemplated by the current invention may also include a pharmaceutically acceptable carrier.

By "pharmaceutically acceptable carrier" is intended, but not limited to, a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type, such as liposomes.

A pharmaceutical composition of the present invention for parenteral injection can comprise pharmaceutically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), carboxymethylcellulose and suitable mixtures thereof, vegetable oils (such as olive oil), and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

The compositions of the present invention can also contain adjuvants such as, but not limited to, preservatives, wetting agents, emulsifying agents, and dispersing agents. Prevention of the action of microorganisms can be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol, sorbic acid, and the like. It can also be desirable to include isotonic agents such as sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, to prolong the effect of the drugs, it is desirable to slow the absorption from subcutaneous or intramuscular injection. This can be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, can depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be

controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium just prior to use.

Solid dosage forms for oral administration include, but are not limited to, capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compounds are mixed with at least one item pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, acetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form can also comprise buffering agents.

Solid compositions of a similar type can also be employed as fillers in soft and hard filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They can optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

The active compounds can also be in micro-encapsulated form, if appropriate, with one or more of the above-mentioned excipients.

Liquid dosage forms for oral administration include, but are not limited to, pharmaceutically acceptable emulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms can contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate,

propylene glycol, 1,3-butylene glycol, dimethyl formamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Suspensions, in addition to the active compounds, can contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar, and tragacanth, and mixtures thereof.

Alternatively, the composition can be pressurized and contain a compressed gas, such as nitrogen or a liquefied gas propellant. The liquefied propellant medium and indeed the total composition is preferably such that the active ingredients do not dissolve therein to any substantial extent. The pressurized composition can also contain a surface active agent. The surface active agent can be a liquid or solid non-ionic surface active agent or can be a solid anionic surface active agent. It is preferred to use the solid anionic surface active agent in the form of a sodium salt.

The compositions of the present invention can also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain, in addition to the compounds of the invention, stabilizers, preservatives, excipients, and the like. The preferred lipids are the phospholipids and the phosphatidyl cholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art (*see*, for example, Prescott, Ed., *Meth. Cell Biol. 14*:33 *et seq* (1976)).

One of ordinary skill will appreciate that effective amounts of the agents of the invention can be determined empirically and can be employed in pure form or, where such forms exist, in pharmaceutically acceptable salt, ester or prodrug form. The agents can be administered to a subject, in need of treatment of viral infection, as pharmaceutical compositions in combination with one or more pharmaceutically acceptable excipients. It will be understood that, when administered to a human patient, the total daily usage of the agents or composition of the present invention will be decided by the attending physician within the scope of sound medical judgement. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors: the type and degree of the cellular or physiological response to be achieved; activity of the specific agent or composition employed; the specific agents or composition employed; the age, body weight, general health, sex and diet of the patient; the time

of administration, route of administration, and rate of excretion of the agent; the duration of the treatment; drugs used in combination or coincidental with the specific agent; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the agents at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosages until the desired effect is achieved.

Dosing can also be arranged in a patient specific manner to provide a predetermined concentration of the agents in the blood, as determined by techniques accepted and routine in the art. Thus patient dosaging can be adjusted to achieve regular on-going blood levels, as measured by HPLC, on the order of from 50 to 1000 ng/ml.

It will be readily apparent to one of ordinary skill in the relevant arts that other suitable modifications and adaptations to the methods and applications described herein can be made without departing from the scope of the invention or any embodiment thereof.

The modified siRNA is prepared by custom chemical synthesis by Dharmacon, at Lafayette CO. Each C and U within the siRNA duplex (GL2), has been substituted with 2'-F-U and 2'-F-C except for the 3'-end overhang, which was dTdT.

To test the stability of 2' chemically modified siRNA compared to unmodified siRNA (siRNA), the following experiment is performed. 4ngs of siRNA are added to a 20 μL volume of 80% human serum from a healthy donor. This mixture is incubated at 37°C for various times ranging from 1 minute up to 10 days. The same process is performed for 2' fluorine modified siRNA (2'-F siRNA). When the incubation process is finished, the mixtures are placed on ice and then immediately separated by PAGE along with a ^{32}P-siRNA control. The 2' modified siRNA is stable as compared to unmodified siRNA.

V. IDENTIFICATION OF THERAPEUTICALLY ACTIVE AGENTS FOR TREATMENT OF SARS VIRUS INFECTION

The invention provides methods for treating SARS by administering therapeutically active agents, such as small molecule compounds, to a mammal, as well as methods of identifying therapeutically active agents, such as potent small molecules, for the treatment of SARS virus infection.

In one aspect of the invention a method of identifying a therapeutically active agent is provided comprising: (a) contacting the therapeutically active agent with a cell infected with the SARS virus; (b) measuring attenuation of a SARS related enzyme.

In a more particular embodiment, the therapeutically active agent is a small molecule. In another more particular embodiment, the therapeutically active agent is a nucleoside analog (*e.g.* Ribavirin). In another more particular embodiment the small molecule is a SMIP or peptidic immunomodulating compound. In another more particular embodiment the therapeutically

active agent is a peptoid, oligopeptide, or polypeptide. In another embodiment the SARS related enzyme is SARS protease. In another embodiment the SARS related enzyme is SARS polymerase. In still another embodiment the SARS related enzyme is a kinase. In still another embodiment, the SARS related enzyme is a protease. The furin inhibitor peptidyl chloromethylketone prevents blocks cell-cell fusion after MHV infection (de Haan *et al.* (2004) *J Virol*), which offers guidance for SARS therapy.

The invention includes a cell-based assay that can be used to screen for and identify a therapeutically active agent for the treatment of SARS virus infection. Therapeutically active agents of the invention include agents that inhibit, prevent or reduce the replication of a SARS virus. Such agents can be identified by infecting a cultured cell (such as, for example, VERO cells) with a SARS virus and evaluating the impact of potential antiviral compounds on SARS virus replication. Assays to measure the effect of a potential antiviral compound on virus replication are known in the art and may be based on a variety of parameters.

The cell-based assay may be used in a high-throughput screen to identify therapeutically active compounds from chemical libraries comprising potential antiviral compounds. Therapeutically active compounds suitable for use in the invention may inhibit any SARS viral target that is essential for viral replication in whole cells. Efficacy (the ability of a compound to inhibit or inactivate the target, be it viral or cellular, that results in the reduction of virus in the culture) of the therapeutic agent is measured by assessing the viability and/or the proliferation of surviving cells in a SARS virus infected cell culture.

A number of methods can be used to measure cell viability are known in the art, such as assays measuring cellular enzymes, proteins, nucleotide triphoshates (*e.g.* ATP), nucleic acids (*e.g.* host cell mRNA (*e.g.* GAPDH) or rRNA sequences) or cellular metabolites such as MTT or MTS. In addition, fluorescent (including, for example HSV paper) or non-fluorescent dyes (*e.g.* propidium diiodide) or labeling of DNA can be used to measure indications of cell viability and/or proliferation.

Alternatively, efficacy of a compound or sample can be determined by directly measuring the amount of virus or viral products in the culture. Methods for measuring the amount of virus, viral genome or viral products include: PCR, RT-PCR, TMA, reporter proteins with fluourescent or luminescent qualities or enzymatic functions (*e.g.*, luciferase, alkaline phosphatase, GFP) or proteins that can be detected by antibodies (*e.g.* EGF) that might be incorporated into the viral genome prior to infection of the cell culture. Further, viral products such as viral proteins can be measured by ELISA or enzymatic activities. Methods for identifying viral polynucleotides, viral proteins and antibodies specific to viral proteins are discussed above.

Potential antiviral compounds are applied to the cell-based assay at a concentration of approximately 10 μM and compound classes having therapeutic effect are identified by

measuring the parameter of choice (such as cell viability/proliferation or the virus or viral genome or a viral product be it viral in origin or non-virus in orgin). Once compounds are identified as having activity, they are resynthesized, and analoged. Starting with the identified compound, many analogs and new compounds are synthesized during consecutive optimization cycles of synthesis, biological profiling and modeling techniques to optimize the to the lead structure until *in vivo* activity is elucidated and optimized.

Cells suitable for use in the assay include the cells described above as suitable for vaccine production. Preferably, the cells are African green monkey kidney cells (Vero) cells. Human embyronic lung fibroblasts or normal human diploid fibroblasts may also be used in the invention.

In one embodiment, the invention includes a fluorescence based cytopathogenicity assay to measure the effect of a potential antiviral compound on a cell-based assay. One example of a fluorescence based cytopathogenicity assay is illustrated below.

1×10^4 Vero cells per well of a microtiter plate (MTP) are infected with a defined amount of SARS virus selected within the following ranges for optimal MOI: 5-10, 10-25, 25-50, 50-100, 100-500, or 500-1000 PFU in a total volume of 200 μl media (M199 medium supplemented with 5% FCS, 2 mM glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin) in the presence or absence of the potential antiviral compound and incubated for at least 1, 2, 3, 4, 5, 6, or 7 days at 37°C, 5% CO_2. The wells of the MTP are washed with PBS (200 μl) and then filled with 200 μl PBS containing 10 μg/ml fluorescein diacetate. After a 45-min incubation at room temperature, fluorescence is measured at 485 nm excitation and 538 nm emission wavelengths. IC_{50} values are determined by a nonlinear plot of antiviral activity as a function of drug concentration.

Other cell based assays are known in the art and include, among others, methods of GFP detection and Luc detection. In addition, a Promega kit is commercially available that provides additional methods of measuring cell viability, *etc.*

In one embodiment, the invention includes a method of measuring the efficacy of a potential antiviral compound using RT-PCR to detect the levels of SARS viral RNA in the cell based assay. Methods of using RT-PCR are known in the art. One example of such an assay is described below.

5×10^6 Vero cells are seeded in tissue culture. Flasks containing the cells are incubated over night at 37°C, 5% CO_2. The cells are infected (m.o.i. = 1) with SARS virus in the presence and absence of potential antiviral compounds. Optionally, the cells may be pretreated with the potential compound prior to infection. In either case, a suitable control cell assay is also prepared.

The RNA of infected cells is purified at 2 h (UL54), 12 h (UL8) and 16 h (UL13) after infection, (Qiagen) RNA purification (RNeasy kit; 40 μl elution) and quantified (absorption at 260 nm). The RNA (2 μg) is reverse transcribed with a specific primer (2 pmol, using one of the primer pairs described herein) into cDNA according to theSuperscript II protocol (Invitrogen). Aliquots (2 μl) of the reverse transcription reaction are amplified by PCR. Fragments of the appropriate target SARS gene, *i.e.*, a gene encoding a SARS enzyme, are amplified in 30 cycles (UL54 and UL8: 3 min, 94°C hot start; 1 min, 94°C denaturation; 1 min, 55°C annealing; 1 min, 72 °C polymerization. UL13: 3 min, 94 °C hot start; 1 min, 94 °C denaturation; 1 min, 60 °C annealing; 1 min, 72°C polymerization) by PCR (Taq-Polymerase, Stratagene), in a 100-μl reaction volume with the appropriate oligonucleotides, as described herein at 0.1 nmol each. 8-μl aliquots of cycle 20–30 (lanes 2–12) of the PCR were resolved on a 2% agarose gel (Invitrogen) according to the manufacturer's instructions.

Cell-based assays of the invention may optionally use a variant or derivative of a wild-type SARS virus that has reduced or attenuated virulence in humans and/or animal models (*e.g.*, mouse, non-human primate, *etc.*) Use of such attenuated SARS viruses in screening methods may reduce safety concerns and precautions that would otherwise be associated with the pathogenic nature of the SARS virus and may eliminate or reduce the need for the implementation of cumbersome high containment levels during performance of the assays and screening of compounds.

The invention includes an enzyme-based assay that can be used to screen for and identify a therapeutically active agent for the treatment of SARS virus infection.

An embodiment of the invention is an assay comprising contacting a known quantity of SARS protease in solution to a peptide containing a detectable marker and cleavage site for SARS protease, wherein SARS protease activity is monitored by measuring the intensity of the marker on the cleaved product.

In a more particular embodiment, a method of assaying for SARS protease is provided comprising contacting a sample solution containing SARS protease with a peptide containing a fluorescent donor, fluorescent quencher, and cleavage site for SARS protease, said peptide being detectable with a fluorometer when cleaved, wherein SARS protease activity is determined in the sample by the amount of fluorescence detected by the fluorometer.

Assays based on the direct measurement of SARS protease inhibition may be utilized for screening for SARS therapeutics. Protease for such assays such as 3C-like protease and papain-like protease may be isolated and purified for such assays as described in Seybert, *et al.*, J. Gen. Virol., 78:71-75, 1997, Ziebuhr, *et al.*, Adv. Exp. Med. Biol., 440:115-120, 1998, Sims, *et al.*, Adv. Exp. Med. Biol. 440:129-134, 1998, Ziebuhr, *et al.*, J. Virol., 73:177-185, 1999, Teng, *et al.*, J. Virol., 73:2658-2666, 1999, Herold, *et al.*, J. Biol. Chem. 274:14918-14925, 1999, and

Ziebuhr, *et al.*, J. Biol. Chem. 276:33220-33232, 2001. Furthermore, Example 30 describes a novel method of purifying SARS protease using column chromatography. Example 31 describes a continuous fluorescence resonance energy transfer (FRET) assay for measuring SARS protease activity. Protease enzyme based assays such as the FRET assay demonstrated in Example 31 are readily adapted for high-throughput screening and are used for screening candidate antiviral compounds. Performance of the protease enzymatic assay in the presence of a SARS protease inhibitor compound will show a decreased amount of fluorescence at a given time when compared to negative control assay containing no test compound on a non-inhibiting control compound. Such a method would involve the steps of: (a) providing an assay solution comprising SARS protease; (b) adding a test compound to the assay solution; (c) adding a substrate for SARS protease to the assay solution; and (d) measuring the proteolytic activity in the assay solution. In a preferred embodiment, the proteolytic activity is measured by the fluorescence of fluorophore product produced by the enzymatic activity of SARS protease.

Attenuated SARS virus variants generally contain one or more genome modifications or mutations (*e.g.*, substitutions, deletions, insertions) in protein encoding or non-coding regions. Specific examples of attenuating mutations include, for example, genetic modifications in the 5'-end noncoding region, leader sequence, intergenic regions, 3'-end noncoding region, ORF 1a, ORF 1b, S gene, E gene, M gene, N gene, or any of the nonstructural protein genes outside of the ORF 1a/1b region. Preferred attenuating mutations are in a SARS virus structural protein (*e.g.*, Spike (S)), a protease or polymerase domain, or a non-coding sequence (*e.g.*, 5'-end noncoding region, intergenic sequence). In addition, a cleavage site may be introduced or eliminated within the spike protein (see for example, Gombold *et al.*, J. Virol. 67:4504-4512, 1993; Bos *et al.*, Virology 214:453-463, 1995), such modification that may also be useful for optimization of expression of recombinant spike protein antigen (*e.g.*, for vaccine purposes).

A variety of methods are used according to the present invention in order to obtain attenuated variants of SARS virus. Such methods include serial passage of the SARS virus in cultured cells (*e.g.*, mammalian cell culture, such as fetal rhesus kidney cells or VERO cells), until the SARS virus demonstrates attenuated function. The serial propagation of virus may be performed at any temperature at which tissue culture passage attenuation occurs, and may be performed in conjunction with one or more steps of mutagenesis (*e.g.*, chemical mutagenesis). The attenuated phenotype of SARS virus variants, obtained after one or more cell culture passages, is readily measured by one skilled in the art. As used herein, attenuation refers to the decreased virulence of the SARS virus in a human subject. Evidence of attenuated function may be indicated by decreased levels of viral replication or by decreased virulence in an animal model.

Other methods of producing an attenuated SARS virus include cell culture passage of the virus at sub-optimal temperatures (cold passage), as well as introduction of attenuating mutations into the SARS viral genome by random mutagenesis (*e.g.*, chemical mutagenesis, such as using 5-fluorouracil) or using directed mutagenesis. Preparation and generation of attenuated RSV vaccines (the methods of which will generally applicable to SARS virus) are disclosed in, for example, EP 0640128, US Patent No. 6,284,254, US Patent No. 5,922,326, US Patent No. 5,882,651.

The number of passages required to obtain safe, immunizing attenuated virus is dependent at least in part on the conditions employed. Periodic testing of the SARS virus culture for virulence and immunizing ability in animals (*e.g.*, mouse, primate) can readily determine the parameters for a particular combination of tissue culture and temperature.

In another embodiment, the cell-based assay for screening of antiviral compounds is based on the readout of expression of a gene product (*e.g.*, reporter gene product) that is not from SARS virus. Gene products particularly suitable to the present invention include, but are not limited to those of the above-described assays.

In order to achieve such a read-out, the gene-of-interest (GOI) encoding said gene reporter gene product must be incorporated into a replicating SARS virus genome or construct derived from a SARS virus genome (*e.g.*, SARS virus replicon, SARS virus defective-interfering (DI) RNA). Figure 13 is a schematic depicting locations for incorporation of the reporter gene into a SARS virus genome. Preferably, insertion of a heterologous reporter gene-of-interest is at a site between existing SARS virus genes, such as for example, as shown in Figure 13. For example, the GOI may be inserted closely following the termination codon of a SARS virus gene (*e.g.*, ORF 1b, S, E, M, N). Insertion should be positioned in order to minimize disruption of mRNA transcription for the SARS virus gene(s). The GOI may also be inserted as an in-frame "fusion" with an existing SARS virus gene, such that sufficient function of the GOI is maintained for detection. To optimize expression, an additional SARS virus intergenic sequence (*e.g.*, SEQ ID NO: 7388, with or without additional flanking SARS virus sequences) may also be engineered into a position preceding the inserted GOI.

Incorporation of a GOI into SARS virus may be accomplished by one of skill in the art using a variety of techniques. For example, one preferred method is targeted RNA recombination, that takes advantage of the ability of coronavirus RNAs to undergo recombination within the cell (see for example Fischer *et al.*, J. Virol. 71:5148-5160, 1997; Koljesar *et al.*, J. Vet. Sci. 2:149-157, 2001). A construct of desired configuration (*e.g.*, cDNA of defective interfering RNA of SARS virus) containing the GOI flanked by SARS virus sequence (*e.g.*, intergenic sequence) is generated such that RNA may be transcribed directly within a eukaryotic cell or in vitro and transfected into susceptible cells also infected with SARS

virus. Recombinant virus containing the GOI is identified based on expression of the GOI encoded marker.

Alternatively, incorporation of a GOI into SARS virus may be accomplished by one of skill in the art by first assembling a full-length cDNA clone of the SARS virus, that can be used to produce infectious RNA transcripts *in vivo* (*e.g.*, from an RNA polymerase II promoter) or *in vitro* (*e.g.*, from a bacteriophage promoter). Although relatively long in genome length, such assembly of a full-length cDNA clone is now readily obtainable by one of skill in the art using standard molecular biology and reverse genetics techniques and the genome sequence of SARS virus (see for example, Thiel *et al.*, J. Gen. Virol., 82:1273-1281, 2001; Almazan *et al.*, Proc. Natl. Acad. Sci. USA 97:5516-5521, 2000; Thiel *et al.* (2003) *J Gen Virol* 82:1273-1281; Yount *et al* (2003) *PNAS USA* 100:12995-13000). Insertion of a heterologous GOI into a full-length SARS virus genome cDNA may be performed using a variety of techniques, such as for example, ligation into natural or synthetic restriction sites, PCR (*e.g.*, overlapping PCR), and recombination.

It may also be desirable to utilize similar SARS virus recombinants containing a gene-of-interest for antiviral screening, however, with further modification to minimize or eliminate virus-induced cytopathology (*e.g.*, CPE). Non-cytopathic derivatives from SARS virus may be obtained by one of skill in the art using a variety of methods. For example, a selectable marker (*e.g.*, drug resistance marker) may be incorporated as GOI into a SARS virus genome to produce infectious virus as described above (see for example, Perri *et al.*, J. Virol., 74:9802-9807, 2000). Infectious GOI-containing SARS virus or infectious genome RNA/cDNA is then used to infect/transfect cells (*e.g.*, VERO), with or without prior mutagenesis, after which time the infected cells are subjected to the appropriate selection. Only those cells containing SARS virus harboring both the selectable marker and one or more mutations rendering the virus non-cytopathic will survive the selection process and grow out. Active SARS virus replication in these cells is readily detected using a variety of detection techniques (*e.g.*, PCR, Northern blot) and such cells may serve as the substrate for cell-based screening assays. Mutations that result in the desired noncytopathic SARS virus phenotype may include nucleotide substitutions, deletions or additions, and may occur in a variety of genome coding or non-coding regions (*e.g.*, 5' or 3'-end noncoding regions, intergenic regions, ORF1a, ORF1b, a protease domain, a polymerase domain). The identification of such mutations is readily accomplished by exchange of sequences with wild-type (*e.g.*, parental) SARS virus and demonstrating transfer of the phenotype, and sequencing of the appropriate genome region. Similar mutations that reduce or eliminate cytopathogenicity also may be utilized in the context of a SARS virus derived replicon vector, either by similar selection directly using a SARS virus replicon or by specific engineering of the replicon based on mutation(s) identified in the context of infectious SARS virus as described

above. In addition, such mutations may serve as the basis for attenuated SARS virus derivatives, as described elsewhere in this document.

Alternatively, rather than using infectious SARS virus or its derivatives for cell-based screening assays, propagation defective "replicons" may be engineered and utilized. Such replicons maintain all protein encoding sequences and cis replication sequences required for RNA replication and expression within a cell, but are deleted of one or more sequences or genes required for packaging of progeny SARS virus (see for example Curtis *et al.*, J. Virol., 76:1422-1434, 2002). Figure 14 is a schematic depicting representative examples of SARS virus replicons according to the present invention. For example a SARS virus cDNA construct is generated, that is lacking one or more (or all) structural protein encoding genes, whereby the missing SARS virus gene(s) is/are replaced by the GOI, maintaining all necessary transcription signals for expression of the GOI. Operably linked with the SARS virus replicon cDNA construct is a promoter for RNA polymerase that can be used to transcribe the replicon RNA *in vivo* (*e.g.*, RNA polymerase II promoter) or in vitro (*e.g.*, bacteriophage promoter). The SARS replicon may be introduced into a susceptible cell by transfection as RNA or DNA, depending on the promoter of choice, and the transfected cells may be utilized for the evaluation of antiviral compounds. By incorporating one or more mutations rendering the replicon noncytopathic for the cells (see above), one can avoid the need for nucleic acid transfection each time an assay is to be performed.

Alternatively, SARS virus replicons may be packaged into virus like particles that allow infection of cells, rather than requiring transfection of nucleic acid molecules. A requirement for replicon packaging is that essential SARS virus gene functions deleted from the replicon (*e.g.*, one or more structural proteins) are provided in *trans* within the cell containing the replicon. A variety of methods for packaging of replicon RNA can be utilized to one of skill in the art (see for example, Curtis *et al.*, ibid: Ortego, *et al.*, J. Virol., 76:11518-11529, 2002). For example, stably transformed cell lines constitutively or inducibly expressing the required SARS virus gene functions may be utilized. Alternatively, the required SARS virus gene functions may be expressed by viral vectors that are introduced into the replicon-containing cell. Alternatively a defective interfering (DI) SARS virus derived RNA containing the required gene functions may be introduced into the replicon-containing cell. Such DI constructs used to complement missing replicon functions may be more commonly referred to as defective helper RNA or defective helpers.

Another configuration useful for cell-based antiviral screening assays according to the present invention utilizes SARS virus derived DI RNAs encoding a GOI (see for example Stirrups, *et al.*, J. Gen. Virol., 81:1687-1698, 2000; Liao, *et al.*, Virology 208:319-327, 1995).

Introduction of a SARS DI, either as cDNA linked to an RNA polymerase II promoter or as in vitro transcribed RNA, into susceptible cells also infected with SARS virus, allows for a readout of the GOI reporter product in assays.

A replicon-based system for rapid identification of coronavirus replicase inhibitors is described by Hertzig *et al.* (2004) *J Gen Virol* DOI 10.1099/vir/0/80044-0. Briefly, the system uses a non-cytopathic selectable replicon RNA that can be stably maintained in eukaryotic cells. The replicon RNA mediates reporter gene expression as a marker for coronavirus replication, and expression of the reporter can be used to test the inhibitory effect of test compounds *in vitro*, thereby allowing high throughput screening for replicase inhibitors without the need to grow infectious virus. Preferred replicon RNAs include a neomycin resistance gene in the replicase gene with a downstream reporter gene (*e.g.* GFP) that is expressed via replicase-mediated synthesis of a sub-genomic mRNA.

VI. COMPOSITIONS AND METHODS FOR TREATMENT OF SARS VIRUS INFECTION

The present invention relates to compositions and methods for the treatment and/or prevention of SARS. The invention further includes a method for the treatment and/or prevention of SARS through the administration of a therapeutically effective amount of at least one antiviral compound from among those described in the US Patents and published international patent applications listed in Table 1 and Table 2. In one embodiment of the method, the antiviral compound is a small molecule. In another embodiment, the antiviral compound is a protease inhibitor. In a further embodiment, the antiviral protease inhibitor is a 3C-like protease inhibitor and/or a papain-like protease inhibitor. Combined treatment with the lopinavir/ritonavir (Kaletra) protease inhibitor and ribavirin has shown a favorable clinical response (Chu *et al.* (2004) *Thorax* 59:252-256). In another embodiment, the antiviral compound is an inhibitor of an RNA dependent RNA polymerase. In another embodiment, a first antiviral compound that is a protease inhibitor is administered with a second antiviral compound that is an RNA-dependent RNA polymerase inhibitor. The invention further provides for the administration of a steroidal anti-inflammatory drug in combination with at least one antiviral compound, for example, from the antiviral compounds described in the documents listed in Table 1 and Table 2. A combination treatment of steroids and ribavirin has been described by Fujii *et al.* (2004) *J Infect Chemother* 10:1-7. A combination treatment of corticosteroids and interferon alfacon-1 has also been reported (Loutfy *et al.* (2003) *JAMA* 290:3222-3228).

The invention further provides for a method for the treatment and/or prevention of SARS through the administration of a therapeutically effective amount of at least one antiviral

compound from among those described in the US Patents and published international patent applications listed in Table 1 and Table 2 by inhalation. In another aspect, the antiviral compound may be administered in combination with a SMIP, SMIS, or other immunomodulatory compound such as those in Table 34 and in Table 35. In one embodiment of the method, the antiviral compound is a small molecule. In another embodiment, the antiviral compound is a protease inhibitor. In a further embodiment, the antiviral protease inhibitor is a 3C-like protease inhibitor and/or a papain-like protease inhibitor. In another embodiment, the antiviral compound is an inhibitor of an RNA dependent RNA polymerase. In another embodiment, a first antiviral compound that is a protease inhibitor is administered with a second antiviral compound that is an RNA-dependent RNA polymerase inhibitor. The invention further provides for the administration of a steroidal anti-inflammatory drug in combination with at least one antiviral compound, for example, from the antiviral compounds described in the documents listed in Table 1 and Table 2. The steroidal anti-inflammatory drug may be administered by inhalation for a local effect or administered for systemic absorption such as via an oral or intravenous route.

The invention further provides for methods for treating SARS infection comprising administering a small molecule immunopotentiator (SMIP) compound either alone or in combination with an antiviral compound or in combination with a SARS vaccine. In a further embodiment, the SMIP is a compound disclosed herein or set forth in Table 34.

The invention further provides for methods for treating SARS infection comprising administering an immunosuppressant compound, optionally a small molecule suppressant (SMIS) compound either alone or in combination with an antiviral compound. In a further embodiment, the immunosuppressant compound is disclosed herein or set forth in Table 35.

The invention further provides peptidic immunomodulating compositions, that include oligo and polypeptides, capable of effecting inflammatory response in a patient. In one embodiment, the peptidic immunomodulating composition is able to stimulate human cells to produce cytokines. In another embodiment the peptidic immunomodulating composition is capable of decreasing cytokine levels in the human. Preferred Examples of peptidic immunomodulating compositions include those listed in Table 35, as well as TGFβ2, TGFβ1, TGFβ3, thymopentin (TP5), β-mercaptopropionyl-arginyl--lysyl-aspartyl-valyl-tyrosyl-cysteine amide, colostrinine, lactoferrin (LF), cyclolinopeptide A (CLA), and tuftsin (TKPR). The peptidic immunomodulating compositions of the invention may be used alone or in combination with other agents, preferably antiviral compounds, for the treatment of SARS.

The invention further provides for a kit for use by a consumer for the treatment and/or prevention of SARS. Such a kit comprises: a) a pharmaceutical composition comprising a therapeutically effective amount of at least one antiviral, SMIP, SMIS, or other immunomodulating compound from among those described in the US Patents and published international patent applications listed in Table 1, Table 2, Table 34 and Table 35 and a pharmaceutically acceptable carrier, vehicle or diluent; b) a container for holding the pharmaceutical composition; and, optionally, c) instructions describing a method of using the pharmaceutical compositions for the treatment and or the prevention of SARS. The kit may optionally contain a plurality of compounds for the treatment of SARS wherein the antiviral compounds are selected from 3C-like protease inhibitors and papain-like protease inhibitors. In a further embodiment, the kit contains an antiviral compound that is an RNA-dependent RNA polymerase inhibitor. When the kit comprises more than one antiviral, SMIP, SMIS, or other immunomodulating compound, the compounds contained in the kit may be optionally combined in the same pharmaceutical composition.

An additional aspect of the invention provides for the use of at least one of the antiviral, SMIP, SMIS, or other immunomodulating compounds described in the US Patents and published international patent applications listed in Table 1, Table 2, Table 34 and Table 35 for the manufacture of a medicament for the treatment or prevention of SARS.

An additional aspect of the invention provides for the use of at least one SMIP compound, or at least one immunosuppressant compound, or at least one SMIS compound for the manufacture of a medicament for the treatment or prevention of SARS. Preferred SMIP, immunosuppressant, and SMIS compounds are described herein.

Unless otherwise specified, the following terms, when used within Section VI: "Compositions and Methods for Treatment of SARS Virus Infection" of the present application have the meanings as defined below:

As used herein, "limit", "treat" and "treatment" are interchangeable terms as are "limiting" and "treating" and, as used herein, include preventative (*e.g.*, prophylactic) and palliative treatment or the act of providing preventative or palliative treatment. The terms include a postponement of development of SARS symptoms and/or a reduction in the severity of such symptoms that will or are expected to develop following infection with a SARS virus. The terms further include ameliorating existing SARS symptoms, preventing additional symptoms, ameliorating or preventing the underlying metabolic causes of symptoms.

Representative uses of the compositions and methods of the present invention include: the elimination or reduction of the viral load of the SARS virus in a vertebrate, including humans, the elimination or reduction of symptoms associated with SARS, and a reduction in morbidity

associated with SARS. In a SARS patient population, the use of the compositions and methods of the invention will result in the reduction in the high mortality rates associated with SARS.

Infection with the SARS virus and the symptoms associated with SARS can be treated in a subject by administering the compositions of the invention. The compositions of the invention may be administered systemically. For systemic use, the compounds herein are formulated for parenteral (*e.g.*, intravenous, subcutaneous, intramuscular, intraperitoneal, intranasal or transdermal) or enteral (*e.g.*, oral or rectal) delivery according to conventional methods. Intravenous administration can be by a series of injections or by continuous infusion over an extended period. Administration by injection or other routes of discretely spaced administration can be performed at intervals ranging from weekly to once to three times daily or more. Alternatively, the compositions disclosed herein may be administered in a cyclical manner (administration of disclosed composition, followed by no administration, followed by administration of disclosed compositions, and the like). Treatment will continue until the desired outcome is achieved.

A "subject" is a vertebrate animal including a human that is in need of treatment with the compositions, methods and kits of the present invention. The term "subject" or "subjects" is intended to refer to both the male and female gender unless one gender is specifically indicated.

"Coadministration" of a combination of a plurality of antiviral compounds means that these components can be administered together as a composition or as part of the same, unitary dosage form. "Co-administration" also includes administering a plurality of antiviral compounds separately but as part of the same therapeutic treatment program or regimen. "Co-administration" also includes administering a plurality of other agents, such as, for example an oligopeptide, a polypeptide, a peptidic immunomodulator, nucleic acid, antibodies, or a vaccine wherein the compounds or agents are administered separately but as part of the same therapeutic treatment program or regimen. The components need not necessarily be administered at essentially the same time, although they can if so desired. "Co-administration" also includes separate administration at different times and in any order. For example, where appropriate a patient may take one or more component(s) of the treatment in the morning and the one or more of the other component(s) at night.

By "antiviral compound" as used herein is meant an antiviral compound as described in the US Patents and published international patent applications listed in Table 1 and Table 2. The US Patents and published international patent applications listed in Table 1, Table 2 and Table 35 are incorporated herein in their entirety. In one embodiment, the antiviral compound is an RNA-dependent RNA polymerase. In another preferred embodiment the antiviral compound is a 3C-like protease inhibitor or a papain-like protease inhibitor. The antiviral compounds may be

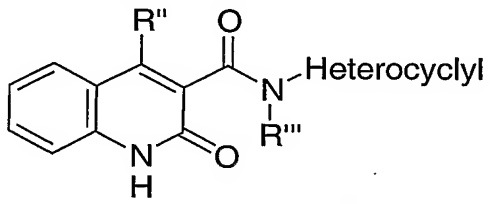
administered in the form of the acid, or of a soluble alkali metal salt or alkaline earth metal salt where appropriate.

The precise dosage of the antiviral compound will vary with the dosing schedule, the oral potency of the particular antiviral compound chosen, the age, size, sex and condition of the subject, the severity of the disorder to be treated, and other relevant medical and physical factors. Thus, a precise pharmaceutically effective amount cannot be specified in advance and can be readily determined by the caregiver or clinician.

Generally, an appropriate amount of antiviral compound is chosen to obtain a reduction in the load of the SARS virus in the subject and/or to obtain a reduction in the symptoms associated with SARS. For humans, an effective oral dose of antiviral compound is typically from about 1.5 to about 6000 μg/kg body weight per day and preferably about 10 to about 2000 μg/kg of body weight per day.

One of ordinary skill in the art will recognize that certain antiviral, SMIP, SMIS, and immunomodulating compounds of the invention including 3C-like protease inhibitors, papain-like protease inhibitators, and RNA-dependent RNA polymerase inhibitors will contain one or more atoms that may be in a particular stereochemical, tautomeric, or geometric configuration, giving rise to stereoisomers, tautomers and configurational isomers. All such isomers and mixtures thereof are included in this invention, when active. Crystalline and amorphous forms of the antiviral compounds of this invention are also included as are hydrates, solvates, polymorphs, and isomorphs of the antiviral compounds of the invention.

SMIP compounds of the invention include compounds are described in issued U.S. Patent Nos. 4,547,511 and 4,738,971 with the general structure (a):

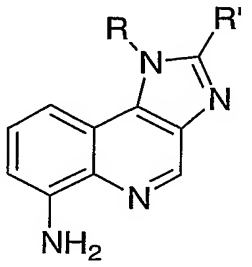


a

for the treatment of disorders responsive to agents that enhance cell-mediated immunity.

Immunostimulatory oligonucleotides and polynucleotides are described in PCT WO 98/55495 and PCT WO 98/16247. U.S. Patent Application No. 2002/0164341 describes adjuvants including an unmethylated CpG dinucleotide (CpG ODN) and a non-nucleic acid adjuvant. U.S. Patent Application No. 2002/0197269 describes compositions comprising an antigen, an antigenic CpG-ODN and a polycationic polymer.

Additionally, issued U.S. Patent Nos. 4,689,338, 5,389,640, 5,268,376, 4,929,624, 5,266,575, 5,352,784, 5,494,916, 5,482,936, 5,346,905, 5,395,937, 5,238,944, 5,525,612, WO99/29693 and U.S. Ser. No. 09/361,544 disclose compounds of the general structure (b):

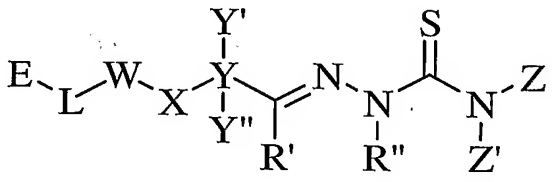


b

for the use as "immune response modifiers."

Further compounds with SMIP and antiviral activity are described below and in US Patent Application entitled Thiosemicarbazones as Anti-Virals and Immunopotentiators filed on December 29, 2003 with an attorney docket number of PP19814.004US generally disclosing compounds of the following structures:

A compound of formula c:



c

wherein: E is absent or selected from the group consisting of alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heterocyclyl, substituted heterocyclyl, heteroaryl, and substituted heteroaryl;

L is absent or is selected from the group consisting of oxo, amino, alkylene, substituted alkylene, alkoxy, alkylamino, aminoalkyl, heterocyclyl, carbocyclyl, and carbonyl;

W is absent or selected from the group consisting of cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heterocyclyl, substituted heterocyclyl, heteroaryl, and substituted heteroaryl;

X is absent or is selected from the group consisting of oxo, amino, alkylene, substituted alkylene, alkoxy, alkylamino, aminoalkyl, heterocyclyl, carbocyclyl, and carbonyl;

Y is selected from the group consisting of cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heterocyclyl, substituted heterocyclyl, heteroaryl, and substituted heteroaryl;

Y' is absent or is selected from the group consisting of F, Cl, Br, I, nitro, alkyl, substituted alkyl, and optionally substituted heterocyclyl, amino, alkylamino, dialkylamino;

Y" is absent or is selected from the group consisting of F, Cl, Br, I, nitro, alkyl, substituted alkyl, and optionally substituted heterocyclyl, amino, alkylamino, dialkylamino;

R' is H, alkyl, or substituted alkyl;

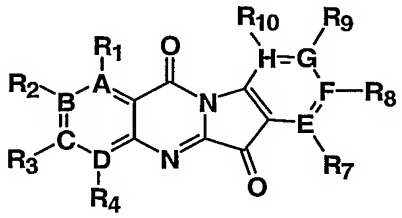
R'' is H, or

R' and R'' are taken together to form a hetercyclic ring;

Z and Z' are independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, alkoxy, substituted alkoxy, aminocarbonyl, alkoxycarbonyl, carboxyl sulfonyl, methanesulfonyl, and substituted or unsubstituted alkylcarbonyl, arylcarbonyl, aralkylcarbonyl, heteroarylcarbonyl, heteroaralkylcarbonyl, alkylcarbonyloxy, arylcarbonyloxy, aralkylcarbonyloxy, heteroarylcarbonyloxy, heteroaralkylcarbonyloxy, alkylaminocarbonyloxy, arylaminocarbonyloxy, formyl, loweralkylcarbonyl, loweralkoxycarbonyl, aminocarbonyl, aminoaryl, alkylsulfonyl, sulfonamido, aminoalkoxy, alkylamino, heteroarylamino, alkylcarbonylamino, alkylaminocarbonylamino, arylaminocarbonylamino, aralkylcarbonylamino, heteroarylcarbonylamino, arylcarbonylamino, cycloamidino, cycloalkyl, cycloimido, arylsulfonyl and arylsulfonamido; or

Z and Z' are taken together to form a heterocyclic group, that may be optionally substituted and the tautomers and the pharmaceutically acceptable salts, esters, or prodrugs thereof.

Further SMIP compounds are described below and in US Patent Application 10/762873, Use of Tryptanthrin Compounds for Immune Potentiation, filed on January 21, 2004 and disclosing the general embodiment of compounds represented by Formula (d):



(d)

wherein

A, B, C, D, E, F, G, and H are independently selected from carbon and nitrogen, or A and B and/or C and D can be taken together to be nitrogen or sulfur;

R_1, R_2, R_3, R_4, R_8, and R_{10} are independently selected from the group consisting of hydrogen, halogen, loweralkyl, alkyl, substituted alkyl, cycloalkyl, heterocyclyl, alkylheterocyclyl, substituted heterocyclyl, substituted alkenyl, amino, (substituted alkyl)(alkyl)amino, imino, halologweralkyl, hydroxy, alkoxy, substituted alkoxy, hydroxyalkylthio, nitro, alkylsulfonyl, N-alkylsulfonamide, arylalkyl, arylalkylaryl, arylaryl, aryloxy, arylamino, acylamino, acyloxyamino, alkylaminoacylamino, alkylaminosulfonylamino, alkylamino, alkenylamino, dialkylamino, alkoxyalkylamino, alkoxyalkylheterocyclyl, mercaptoalkoxyalkyl, cyano, formyl, -$COOR_{11}$ wherein R_{11} is hydrogen, loweralkyl, aryl, heterocyclyl, monosaccharide or disaccharide, and -$CONR_{12}R_{13}$ wherein R_{12} and R_{13} are independently selected from hydrogen, loweralkyl, aryl, heterocyclyl, saccharide, peptide and amino acid residues; or R_2 and R_3 taken together form a six membered aromatic ring;

R_7 and R_9 are independently selected from hydrogen, halogen, loweralkyl, halologweralkyl, cycloalkyl, heterocyclyl, substituted heterocyclyl or heterocyclylalkyl; and

R_1, R_2, R_3, R_4, R_7, R_8, R_9,and R_{10} are absent when the ring atom to which they would otherwise be bonded is sulfur or double-bonded nitrogen; or

the a pharmaceutically acceptable salts, esters, or prodrugs thereof,

provided that R_1, R_2, R_3, R_4, R_7, R_8, R_9, and R_{10} are not all hydrogen when A, B, C, D, E, F, and H are carbon.

In one embodiment, the compounds of Formula (I) have a backbone structure wherein D is nitrogen, and A-C and E-H are carbon.

In one embodiment, when D is carbon, at least one, or at least two of R_1 - R_4, and R_7 - R_{10} are not hydrogen.

In one embodiment, R_1 through R_4, and R_8 and R_{10} are independently selected from at least two of the group consisting of hydrogen, halogen, loweralkyl, cycloalkyl, heterocyclyl, substituted heterocyclyl, alkylheterocyclyl, amino, imino, halologweralkyl, alkoxy, nitro, alkylsulfonyl, arylalkyl, arylalkylaryl, arylaryl, aryloxy, arylamino, acylamino, acyloxyamino, alkylaminoacylamino, alkylaminosulfonylamino, alkylamino, alkenylamino, dialkylamino, alkoxyalkylamino, alkoxyalkylheterocyclyl, mercaptoalkoxyalkyl, cyano, formyl, -$COOR_{11}$ where R_{11} is hydrogen, loweralkyl, aryl, heterocyclyl, monosaccharide or disaccharide, and -$CONR_{12}R_{13}$ where R_{12} and R_{13} are independently selected from hydrogen, loweralkyl, aryl, heterocyclyl, saccharide, peptide and amino acid residues; and R_4 is not present when D is nitrogen.

In an additional embodiment, 4A, B, C, D, E, F, G, and H are independently selected from carbon and nitrogen;

R_1, R_2, R_3, R_4, R_8 and R_{10} are independently selected from the group consisting of hydrogen, halogen, loweralkyl, alkyl, substituted alkyl, heterocyclyl, substituted heterocyclyl, substituted alkenyl, (substituted alkyl)(alkyl)amino, halologweralkyl, hydroxy, alkoxy, substituted alkoxy, hydroxyalkylthio, nitro, N-alkylsulfonamide, cyano, -$COOR_{11}$ wherein R_{11} is hydrogen, loweralkyl, aryl, heterocyclyl, monosaccharide or disaccharide, and -$CONR_{12}R_{13}$ wherein R_{12} and R_{13} are independently selected from hydrogen, loweralkyl, aryl, heterocyclyl, saccharide, peptide and amino acid residues.

For the compounds described herein:

The term "loweralkyl" refers to branched or straight chain acyclical alkyl groups comprising one to ten carbon atoms, including, e.g., methyl, ethyl, propyl, isopropyl, n-butyl, t-butyl, neopentyl and the like.

The term "alkyl" refers to alkyl groups that do not contain heteroatoms. Thus the term includes straight chain alkyl groups such as methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl and the like. The phrase also includes branched chain isomers of straight chain alkyl groups, including but not limited to, the following that are provided by way of example: –$CH(CH_3)_2$, -$CH(CH_3)(CH_2CH_3)$, -$CH(CH_2CH_3)_2$, -$C(CH_3)_3$, -$C(CH_2CH_3)_3$, -$CH_2CH(CH_3)_2$, -$CH_2CH(CH_3)(CH_2CH_3)$, -$CH_2CH(CH_2CH_3)_2$, -$CH_2C(CH_3)_3$, -$CH_2C(CH_2CH_3)_3$, -$CH(CH_3)CH(CH_3)(CH_2CH_3)$, -$CH_2CH_2CH(CH_3)_2$, -$CH_2CH_2CH(CH_3)(CH_2CH_3)$, -$CH_2CH_2CH(CH_2CH_3)_2$, -$CH_2CH_2C(CH_3)_3$, -$CH_2CH_2C(CH_2CH_3)_3$, -$CH(CH_3)CH_2CH(CH_3)_2$, -$CH(CH_3)CH(CH_3)CH(CH_3)_2$, -$CH(CH_2CH_3)CH(CH_3)CH(CH_3)(CH_2CH_3)$, and others. The term also includes cyclic alkyl groups such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl and such rings substituted with straight and branched chain alkyl groups as defined above. The term also includes polycyclic alkyl groups such as, but not limited to, adamantyl norbornyl, and bicyclo[2.2.2]octyl and such rings substituted with straight and branched chain alkyl groups as defined above. Thus, the phrase unsubstituted alkyl groups includes primary alkyl groups, secondary alkyl groups, and tertiary alkyl groups. Unsubstituted alkyl groups may be bonded to one or more carbon atom(s), oxygen atom(s), nitrogen atom(s), and/or sulfur atom(s) in the parent compound. Preferred unsubstituted alkyl groups include straight and branched chain alkyl

groups and cyclic alkyl groups having 1 to 20 carbon atoms. More preferred such unsubstituted alkyl groups have from 1 to 10 carbon atoms while even more preferred such groups have from 1 to 5 carbon atoms. Most preferred unsubstituted alkyl groups include straight and branched chain alkyl groups having from 1 to 3 carbon atoms and include methyl, ethyl, propyl, and –$CH(CH_3)_2$.

The phrase "substituted alkyl" refers to an unsubstituted alkyl group as defined above in which one or more bonds to a carbon(s) or hydrogen(s) are replaced by a bond to non-hydrogen and non-carbon atoms such as, but not limited to, a halogen atom in halides such as F, Cl, Br, and I; a phosphorus atom in groups such as phosphate and dialkyl alkylphosphonate; oxygen atom in groups such as hydroxyl groups, alkoxy groups, aryloxy groups, and ester groups; a sulfur atom in groups such as thiol groups, alkyl and aryl sulfide groups, sulfone groups, sulfonyl groups, and sulfoxide groups; a nitrogen atom in groups such as amines, amides, alkylamines, dialkylamines, arylamines, alkylarylamines, diarylamines, N-oxides, imides, and enamines; a silicon atom in groups such as in trialkylsilyl groups, dialkylarylsilyl groups, alkyldiarylsilyl groups, and triarylsilyl groups; and other heteroatoms in various other groups. Substituted alkyl groups also include groups in which one or more bonds to a carbon(s) or hydrogen(s) atom is replaced by a bond to a heteroatom such as oxygen in carbonyl, carboxyl, and ester groups; nitrogen in groups such as imines, oximes, hydrazones, and nitriles. Preferred substituted alkyl groups include, among others, alkyl groups in which one or more bonds to a carbon or hydrogen atom is/are replaced by one or more bonds to fluorine atoms. One example of a substituted alkyl group is the trifluoromethyl group and other alkyl groups that contain the trifluoromethyl group. Other alkyl groups include those in which one or more bonds to a carbon or hydrogen atom is replaced by a bond to an oxygen atom such that the substituted alkyl group contains a hydroxyl, alkoxy, aryloxy group, or heterocyclyloxy group. Still other alkyl groups include alkyl groups that have an amine, alkylamine, dialkylamine, arylamine, (alkyl)(aryl)amine,diarylamine, heterocyclylamine, (alkyl)(heterocyclyl)amine, (aryl)(heterocyclyl)amine, or diheterocyclylamine group.

The term "alkoxy" refers to RO- wherein R, for example, is alkyl such as loweralkyl defined above. Representative examples of loweralkyl alkoxy groups include methoxy, ethoxy, t-butoxy and the like.

The phrase "substituted alkoxy" refers to RO-, where R is, for example, an alkyl substituted, for example, with a halogen. RO is for example OCF_3.

The term "alkenyl" refers to a branched or straight chain groups comprising two to twenty carbon atoms that also comprises one or more carbon-carbon double bonds. Representative alkenyl groups include prenyl, 2-propenyl (i.e., allyl), 3-methyl-2-butenyl, 3,7-dimethyl-2,6-octadienyl, 4,8-dimethyl-3,7-nonadienyl, 3,7,11-trimethyl-2,6,10-dodecatrienyl and the like.

The phrase "substituted alkenyl" refers to alkenyl groups that are substituted, for example, diethyl hex-5-enylphosponate, and others with an alkyl or substituted alkyl group such as dialkyl phosphate or an ester such as an acetate ester.

The phrase "dialkyl amino" refers to an amino group substituted with two alkyl groups such as C1-20 alkyl groups.

The phrase "substituted dialkyl amino" refers to a dialkylamino substituted, for example, with a carboxylic acid, ester, hydroxy or alkoxy.

The term "hydroxyalkylthio" refers to a thio radical to which is appended a hydroxyalkyl group, where the alkyl is for example lower alkyl. An example is hydroxyethylthio, -SCH_2CH_2OH.

The term "N-alkylsulfonamide" refers to the group -SO_2NHalkyl, where alkyl is, for example, octyl.

The term "alkynyl" refers to a branched or straight chain comprising two to twenty carbon atoms that also comprises one or more carbon-carbon triple bonds. Representative alkynyl groups include ethynyl, 2-propynyl (propargyl), 1-propynyl and the like.

The term "aryl" refers to aryl groups that do not contain heteroatoms. Thus the term includes, but is not limited to, groups such as phenyl, biphenyl, anthracenyl, naphthenyl by way of example. Although the phrase "unsubstituted aryl" includes groups containing condensed rings such as naphthalene, it does not include aryl groups that have other groups such as alkyl or halo groups bonded to one of the ring members, as aryl groups such as tolyl are considered herein to be substituted aryl groups as described below. A preferred unsubstituted aryl group is phenyl. Unsubstituted aryl groups may be bonded to one or more carbon atom(s), oxygen atom(s), nitrogen atom(s), and/or sulfur atom(s) in the parent compound, however.

The phrase "substituted aryl group" has the same meaning with respect to aryl groups that substituted alkyl groups had with respect to alkyl groups. However, a substituted aryl group also includes aryl groups in which one of the aromatic carbons is bonded to one of the non-carbon or non-hydrogen atoms described above and also includes aryl groups in which one or more aromatic carbons of the aryl group is bonded to a substituted and/or unsubstituted alkyl, alkenyl,

or alkynyl group as defined herein. This includes bonding arrangements in which two carbon atoms of an aryl group are bonded to two atoms of an alkyl, alkenyl, or alkynyl group to define a fused ring system (e.g. dihydronaphthyl or tetrahydronaphthyl). Thus, the phrase "substituted aryl" includes, but is not limited to tolyl, and hydroxyphenyl among others.

The term "arylalkyl" refers to a loweralkyl radical to which is appended an aryl group. Representative arylalkyl groups include benzyl, phenylethyl, hydroxybenzyl, fluorobenzyl, fluorophenylethyl and the like.

The phrase "unfused arylaryl" refers to a group or substituent to which two aryl groups, that are not condensed to each other, are bound. Exemplary unfused arylaryl compounds include, for example, phenylbenzene, diphenyldiazene, 4-methylthio-1-phenylbenzene, phenoxybenzene, (2-phenylethynyl)benzene, diphenyl ketone, (4-phenylbuta-1,3-diynyl)benzene, phenylbenzylamine, (phenylmethoxy)benzene, and the like. Preferred substituted unfused arylaryl groups include: 2-(phenylamino)-N-[4-(2-phenylethynyl)phenyl]acetamide, 1,4-diphenylbenzene, N-[4-(2-phenylethynyl)phenyl]-2-[benzylamino]acetamide, 2-amino-N-[4-(2-phenylethynyl)phenyl]propanamide, 2-amino-N-[4-(2-phenylethynyl)phenyl]acetamide, 2-(cyclopropylamino)-N-[4-(2-phenylethynyl)phenyl]acetamide, 2-(ethylamino)-N-[4-(2-phenylethynyl)phenyl]acetamide, 2-[(2-methylpropyl)amino]-N-[4-(2-phenylethynyl)phenyl]acetamide, 5-phenyl-2H-benzo[d]1,3-dioxolene, 2-chloro-1-methoxy-4-phenylbenzene, 2-[(imidazolylmethyl)amino]-N-[4-(2-phenylethynyl)phenyl]acetamide, 4-phenyl-1-phenoxybenzene, N-(2-aminoethyl)[4-(2-phenylethynyl)phenyl]carboxamide, 2-{[(4-fluorophenyl)methyl]amino}-N-[4-(2-phenylethynyl)phenyl]acetamide, 2-{[(4-methylphenyl)methyl]amino}-N-[4-(2-phenylethynyl)phenyl]acetamide, 4-phenyl-1-(trifluoromethyl)benzene, 1-butyl-4-phenylbenzene, 2-(cyclohexylamino)-N-[4-(2-phenylethynyl)phenyl]acetamide, 2-(ethylmethylamino)-N-[4-(2-phenylethynyl)phenyl]acetamide, 2-(butylamino)-N-[4-(2-phenylethynyl)phenyl]acetamide, N-[4-(2-phenylethynyl)phenyl]-2-(4-pyridylamino)acetamide, N-[4-(2-phenylethynyl)phenyl]-2-(quinuclidin-3-ylamino)acetamide, N-[4-(2-phenylethynyl)phenyl]pyrrolidin-2-ylcarboxamide, 2-amino-3-methyl-N-[4-(2-phenylethynyl)phenyl]butanamide, 4-(4-phenylbuta-1,3-diynyl)phenylamine, 2-(dimethylamino)-N-[4-(4-phenylbuta-1,3-diynyl)phenyl]acetamide, 2-(ethylamino)-N-[4-(4-phenylbuta-1,3-diynyl)phenyl]acetamide, 4-ethyl-1-phenylbenzene, 1-[4-(2-phenylethynyl)phenyl]ethan-1-one, N-(1-carbamoyl-2-hydroxypropyl)[4-(4-phenylbuta-1,3-diynyl)phenyl]carboxamide, N-[4-(2-phenylethynyl)phenyl]propanamide, 4-methoxyphenyl phenyl ketone, phenyl-N-benzamide, (tert-butoxy)-N-[(4-phenylphenyl)methyl]carboxamide, 2-

(3-phenylphenoxy)ethanehydroxamic acid, 3-phenylphenyl propanoate, 1-(4-ethoxyphenyl)-4-methoxybenzene, and [4-(2-phenylethynyl)phenyl]pyrrole.

The phrase "unfused heteroarylaryl" refers to a unfused arylaryl group where one of the aryl groups is a heteroaryl group. Exemplary heteroarylaryl groups include, for example, 2-phenylpyridine, phenylpyrrole, 3-(2-phenylethynyl)pyridine, phenylpyrazole, 5-(2-phenylethynyl)-1,3-dihydropyrimidine-2,4-dione, 4-phenyl-1,2,3-thiadiazole, 2-(2-phenylethynyl)pyrazine, 2-phenylthiophene, phenylimidazole, 3-(2-piperazinylphenyl)furan, 3-(2,4-dichlorophenyl)-4-methylpyrrole, and the like. Preferred substituted unfused heteroarylaryl groups include: 5-(2-phenylethynyl)pyrimidine-2-ylamine, 1-methoxy-4-(2-thienyl)benzene, 1-methoxy-3-(2-thienyl)benzene, 5-methyl-2-phenylpyridine, 5-methyl-3-phenylisoxazole, 2-[3-(trifluoromethyl)phenyl]furan, 3-fluoro-5-(2-furyl)-2-methoxy-1-prop-2-enylbenzene, (hydroxyimino)(5-phenyl(2-thienyl))methane, 5-[(4-methylpiperazinyl)methyl]-2-phenylthiophene, 2-(4-ethylphenyl)thiophene, 4-methylthio-1-(2-thienyl)benzene, 2-(3-nitrophenyl)thiophene, (tert-butoxy)-N-[(5-phenyl(3-pyridyl))methyl]carboxamide, hydroxy-N-[(5-phenyl(3-pyridyl))methyl]amide, 2-(phenylmethylthio)pyridine, and benzylimidazole.

The phrase "unfused heteroarylheteroaryl" refers to an unfused arylaryl group where both of the aryl groups is a heteroaryl group. Exemplary heteroarylheteroaryl groups include, for example, 3-pyridylimidazole, 2-imidazolylpyrazine, and the like. Preferred substituted unfused heteroarylheteroaryl groups include: 2-(4-piperazinyl-3-pyridyl)furan, diethyl(3-pyrazin-2-yl(4-pyridyl))amine, and dimethyl{2-[2-(5-methylpyrazin-2-yl)ethynyl](4-pyridyl)}amine.

The phrase "fused arylaryl" refers to an aryl group as previously defined that is condensed, and fully conjugated to an aryl group. Representative fused arylaryl groups include biphenyl, 4-(1-naphthyl)phenyl, 4-(2-naphthyl)phenyl and the like.

The phrase "fused heteroarylaryl" refers to an aryl group as previously defined that is condensed, and fully conjugated with a heteroaryl group. Representative fused heteroarylaryl groups include quinoline, quinazoline and the like.

The phrase "fused heteroarylheteroaryl" refers to a heteroaryl group as previously defined that is condensed, and fully conjugated with another heteroaryl group. Representative fused heteroarylheteroaryl groups include pyrazalopyrimidine, imidazoquinoline and the like.

The term "aryloxy" refers to RO- wherein R is an aryl group. Representative arylalkoxy group include benzyloxy, phenylethoxy and the like.

The term "arylalkoxy" refers to a lower alkoxy radical to which is appended an aryl group. Representative arylalkoxy group include benzyloxy, phenylethoxy and the like.

The term "aryloxyaryl" refers to an aryl radical to which is appended an aryloxy group. Representative aryloxyaryl groups include 4-phenoxyphenyl, 3-phenoxyphenyl, 4-phenoxy-1-naphthyl, 3-phenoxy-1-naphthyl and the like.

The term "aryloxyarylalkyl" refers to an arylalkyl radical to which is appended an aryloxy group. Representative aryloxyarylalkyl groups include 4-phenoxyphenylmethyl, 3-phenoxyphenylmethyl, 4-phenoxyphenylethyl, 3-phenoxy-phenylethyl and the like.

The term "arylalkoxyaryl" refers to an aryl radical to which is appended an arylalkoxy group. Representative arylalkoxyaryl groups include 4-benzyloxylphenyl, 3-benzyloxyphenyl and the like.

The term "arylalkoxyarylalkyl" refers to an arylalkyl radical to which is appended an arylalkoxy group. Representative arylalkoxyarylalkyl groups include 4-benzyloxylbenzyl, 3-benzyloxybenzyl and the like.

The term "cycloalkyl" refers to an alicyclic group comprising from 3 to 7 carbon atoms including, but not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and the like.

The term "cycloalkylalkyl" refers to a loweralkyl radical to which is appended a cycloalkyl group. Representative examples of cycloalkylalkyl include cyclopropylmethyl, cyclohexylmethyl, 2-(cyclopropyl)ethyl and the like.

The term "halogen" refers to iodine, bromine, chlorine or fluorine; "halo" refers to iodo, bromo, chloro or fluoro.

The term "haloalkyl" refers to a lower alkyl radical, as defined above, bearing at least one halogen substituent, for example, chloromethyl, fluoroethyl or trifluoromethyl and the like.

The term "heterocyclyl" (or heterocyclic, or heterocyclo) refers to both aromatic and nonaromatic ring compounds including monocyclic, bicyclic, and polycyclic ring compounds such as, but not limited to, quinuclidyl, containing 3 or more ring members of which one or more is a heteroatom such as, but not limited to, N, O, and S. Although the phrase "unsubstituted heterocyclyl" includes condensed heterocyclic rings such as benzimidazolyl, it does not include heterocyclyl groups that have other groups such as alkyl or halo groups bonded to one of the ring members as compounds such as 2-methylbenzimidazolyl are substituted heterocyclyl groups. Examples of heterocyclyl groups include, but are not limited to: unsaturated 3 to 8 membered rings containing 1 to 4 nitrogen atoms such as, but not limited to pyrrolyl, pyrrolinyl, imidazolyl, pyrazolyl, pyridyl, dihydropyridyl, pyrimidyl, pyrazinyl, pyridazinyl, triazolyl (e.g. 4H-1,2,4-triazolyl, 1H-1,2,3-triazolyl, 2H-1,2,3-triazolyl etc.), tetrazolyl, (e.g. 1H-tetrazolyl, 2H tetrazolyl, etc.); saturated 3 to 8 membered rings containing 1 to 4 nitrogen atoms such as, but

not limited to, pyrrolidinyl, imidazolidinyl, piperidinyl, piperazinyl; condensed unsaturated heterocyclic groups containing 1 to 4 nitrogen atoms such as, but not limited to, indolyl, isoindolyl, indolinyl, indolizinyl, benzimidazolyl, quinolyl, isoquinolyl, indazolyl, benzotriazolyl; unsaturated 3 to 8 membered rings containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms such as, but not limited to, oxazolyl, isoxazolyl, oxadiazolyl (e.g. 1,2,4-oxadiazolyl, 1,3,4-oxadiazolyl, 1,2,5-oxadiazolyl, etc.); saturated 3 to 8 membered rings containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms such as, but not limited to, morpholinyl; unsaturated condensed heterocyclic groups containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms, for example, benzoxazolyl, benzoxadiazolyl, benzoxazinyl (e.g. 2H-1,4-benzoxazinyl etc.); unsaturated 3 to 8 membered rings containing 1 to 3 sulfur atoms and 1 to 3 nitrogen atoms such as, but not limited to, thiazolyl, isothiazolyl, thiadiazolyl (e.g. 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,3,4-thiadiazolyl, 1,2,5-thiadiazolyl, etc.); saturated 3 to 8 membered rings containing 1 to 2 sulfur atoms and 1 to 3 nitrogen atoms such as, but not limited to, thiazolodinyl; saturated and unsaturated 3 to 8 membered rings containing 1 to 2 sulfur atoms such as, but not limited to, thienyl, dihydrodithiinyl, dihydrodithionyl, tetrahydrothiophene, tetrahydrothiopyran; unsaturated condensed heterocyclic rings containing 1 to 2 sulfur atoms and 1 to 3 nitrogen atoms such as, but not limited to, benzothiazolyl, benzothiadiazolyl, benzothiazinyl (e.g. 2H-1,4-benzothiazinyl, etc.), dihydrobenzothiazinyl (e.g. 2H-3,4-dihydrobenzothiazinyl, etc.), unsaturated 3 to 8 membered rings containing oxygen atoms such as, but not limited to furyl; unsaturated condensed heterocyclic rings containing 1 to 2 oxygen atoms such as benzodioxolyl (e.g. 1,3-benzodioxoyl, etc.); unsaturated 3 to 8 membered rings containing an oxygen atom and 1 to 2 sulfur atoms such as, but not limited to, dihydrooxathiinyl; saturated 3 to 8 membered rings containing 1 to 2 oxygen atoms and 1 to 2 sulfur atoms such as 1,4-oxathiane; unsaturated condensed rings containing 1 to 2 sulfur atoms such as benzothienyl, benzodithiinyl; and unsaturated condensed heterocyclic rings containing an oxygen atom and 1 to 2 oxygen atoms such as benzoxathiinyl. Heterocyclyl group also include those described above in which one or more S atoms in the ring is double-bonded to one or two oxygen atoms (sulfoxides and sulfones). For example, heterocyclyl groups include tetrahydrothiophene, tetrahydrothiophene oxide, and tetrahydrothiophene 1,1-dioxide. Preferred heterocyclyl groups contain 5 or 6 ring members. More preferred heterocyclyl groups include morpholine, piperazine, piperidine, pyrrolidine, imidazole, pyrazole, 1,2,3-triazole, 1,2,4-triazole, tetrazole, thiomorpholine, thiomorpholine in which the S atom of the thiomorpholine is bonded to one or

more O atoms, pyrrole, homopiperazine, oxazolidin-2-one, pyrrolidin-2-one, oxazole, quinuclidine, thiazole, isoxazole, furan, and tetrahydrofuran.

The phrase "substituted heterocyclyl" refers to an heterocyclyl group as defined above in which one of the ring members is bonded to a non-hydrogen atom such as described above with respect to substituted alkyl groups and substituted aryl groups. Examples, include, but are not limited to, 2-methylbenzimidazolyl, 5-methylbenzimidazolyl, 5-chlorobenzthiazolyl, 1-methyl piperazinyl, and 2-chloropyridyl among others.

"Aminosulfonyl" refers to the group $-S(O)_2-NH_2$. "Substituted aminosulfonyl" refersto the group $-S(O)_2-NRR'$ where R is loweralkyl and R' is hydrogen or a loweralkyl. The term "aralkylaminosulfonlyaryl" refers to the group –aryl-$S(O)_2$–NH-aralkyl, where the aralkyl is loweraralkyl.

"Carbonyl" refers to the divalent group –C(O)-.

"Carbonyloxy" refers generally to the group –C(O)-O-,. Such groups include esters, -C(O)-O-R, where R is loweralkyl, cycloalkyl, aryl, or loweraralkyl. The term "carbonyloxycycloalkyl" refers generally to both an "carbonyloxycarbocycloalkyl" and an "carbonyloxyheterocycloalkyl", i.e., where R is a carbocycloalkyl or heterocycloalkyl, respectively. The term "arylcarbonyloxy" refers to the group –C(O)-O-aryl, where aryl is a mono- or polycyclic, carbocycloaryl or heterocycloaryl. The term "aralkylcarbonyloxy" refers to the group –C(O)-O-aralkyl, where the aralkyl is loweraralkyl.

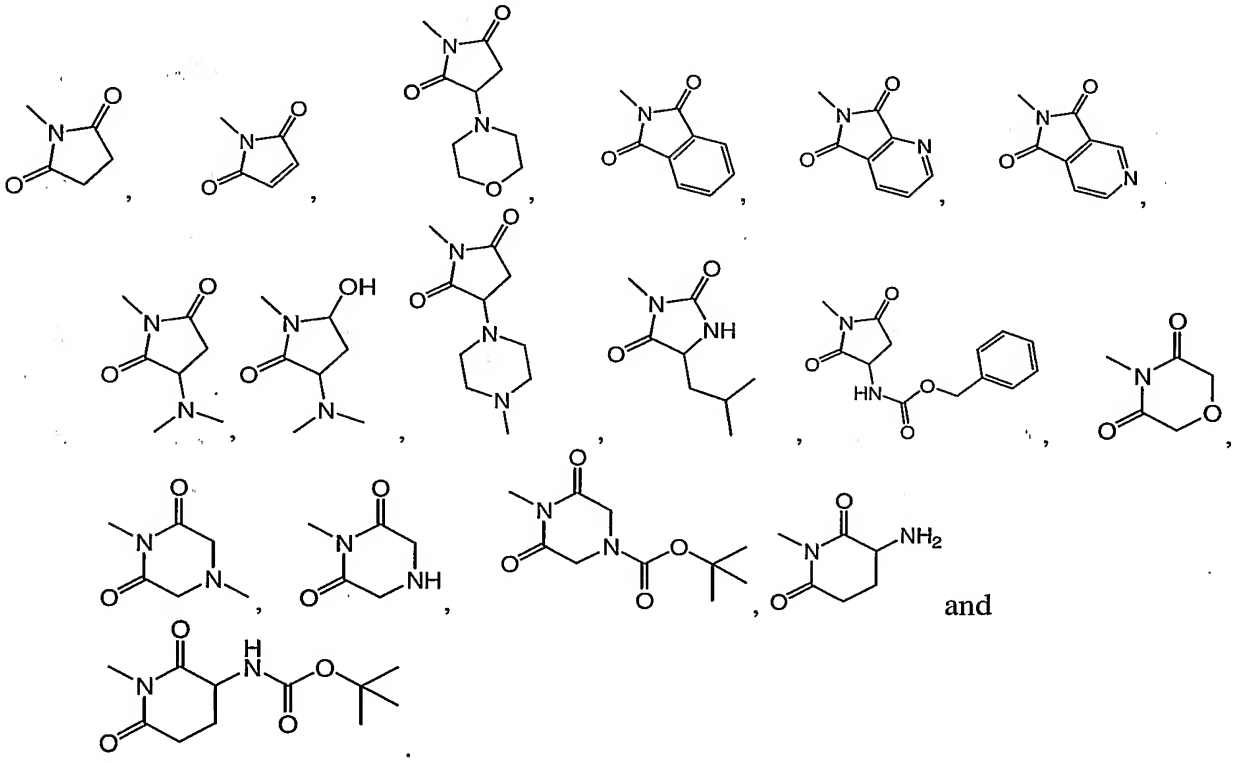
The term "sulfonyl" refers to the group $-SO_2-$. "Alkylsulfonyl" refers to a substituted sulfonyl of the structure $-SO_2R$ - in which R is alkyl. Alkylsulfonyl groups employed in compounds of the present invention are typically loweralkylsulfonyl groups having from 1 to 6 carbon atoms in its backbone structure. Thus, typical alkylsulfonyl groups employed in compounds of the present invention include, for example, methylsulfonyl (i.e., where R is methyl), ethylsulfonyl (i.e., where R is ethyl), propylsulfonyl (i.e., where R is propyl), and the like. The term "arylsulfonyl" refersto the group $-SO_2$-aryl. The term "aralkylsulfonyl" refers to the group $-SO_2$-aralkyl, in which the aralkyl is loweraralkyl. The term "sulfonamido" refers to $-SO_2NH2$.

The term "carbonylamino" refers to the divalent group -NH-C(O)- in which the hydrogen atom of the amide nitrogen of the carbonylamino group can be replaced a loweralkyl, aryl, or loweraralkyl group. Such groups include moieties such as carbamate esters (-NH-C(O)-O-R) and amides –NH-C(O)-O-R, where R is a straight or branched chain loweralkyl, cycloalkyl, or aryl or loweraralkyl. The term "loweralkylcarbonylamino" refers to alkylcarbonylamino where R is a

loweralkyl having from 1 to about 6 carbon atoms in its backbone structure. The term "arylcarbonylamino" refers to group –NH-C(O)-R where R is an aryl. Similarly, the term "aralkylcarbonylamino " refers to carbonylamino where R is a lower aralkyl.

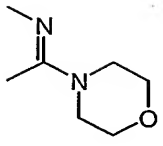
The term "guanidino" or "guanidyl" refers to moieties derived from guanidine, H2N-C(=NH)-NH_2. Such moieties include those bonded at the nitrogen atom carrying the formal double bond (the "2"-position of the guanidine, e.g., diaminomethyleneamino, $(H2N)_2C$=NH-) and those bonded at either of the nitrogen atoms carrying a formal single bond (the "1-" and/or "3"-positions of the guandine, e.g., H_2N-C(=NH)-NH-). The hydrogen atoms at any of the nitrogens can be replaced with a suitable substituent, such as loweralkyl, aryl, or loweraralkyl.

Representative cycloimido and heterocycloimido groups include, for example, those shown below. These cycloimido and heterocycloimido can be further substituted and may be attached at various positions as will be apparent to those having skill in the organic and medicinal chemistry arts in conjunction with the disclosure herein.



and

Representative substituted amidino and heterocycloamidino groups include, for example, those shown below. These amidino and heterocycloamidino groups can be further substituted as will be apparent to those having skill in the organic and medicinal chemistry arts in conjunction with the disclosure herein.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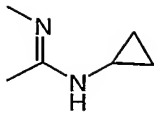
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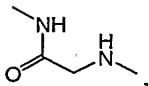
Representative substituted alkylcarbonylamino, alkyloxycarbonylamino, aminoalkyloxycarbonylamino, and arylcarbonylamino groups include, for example, those shown below. These groups can be further substituted as will be apparent to those having skill in the organic and medicinal chemistry arts in conjunction with the disclosure herein.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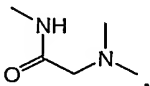
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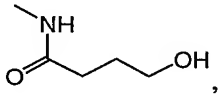
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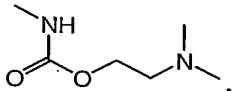
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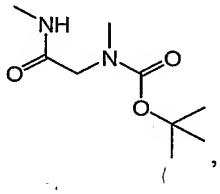
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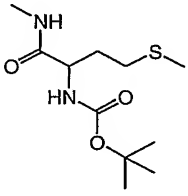
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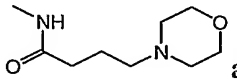
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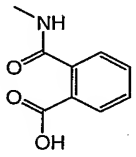
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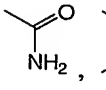
Representative substituted aminocarbonyl groups include, for example, those shown below. These can heterocyclo groups be further substituted as will be apparent to those having skill in the organic and medicinal chemistry arts in conjunction with the disclosure herein.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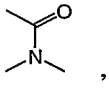
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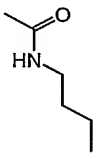
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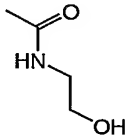
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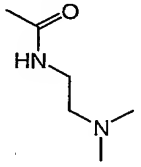
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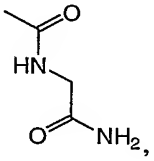
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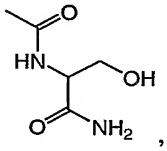
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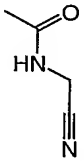
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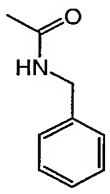
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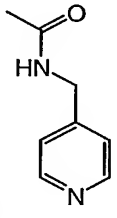
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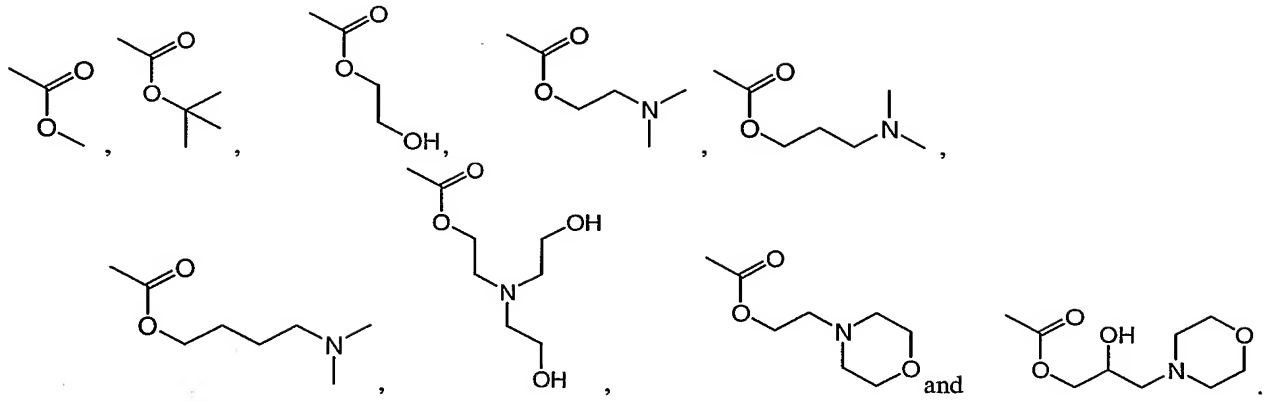
Representative substituted alkoxycarbonyl groups include, for example, those shown below. These alkoxycarbonyl groups can be further substituted as will be apparent to those having skill in the organic and medicinal chemistry arts in conjunction with the disclosure herein.



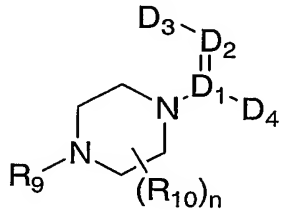
"Substituted" refers to the definite replacement of hydrogen with one or more monovalent or divalent radicals. Suitable substitution groups include, those described herein for particular groups, as well as hydroxyl, nitro, amino, imino, cyano, halo, thio, thioamido, amidino, imidino, oxo, oxamidino, methoxamidino, imidino, guanidino, sulfonamido, carboxyl, formyl, alkyl, substituted alkyl, halologweralkyl, loweralkoxy, halologweralkoxy, loweralkoxy-alkyl, alkylcarbonyl, arylcarbonyl, aralkylcarbonyl, heteroarylcarbonyl, heteroaralkylcarbonyl, alkylthio, aminoalkyl, cyanoalkyl, benzyl, pyridyl, pyrazolyl, pyrrole, thiophene, imidazolyl, and the like.

The term "linking moiety" refers to a covalent bond or an uncyclized divalent group, such as, for example, -CO-, -O-, -S-, -CH_2-, -NH-, and substituted or unsubstituted alkyl, alkenyl, alkynyl, carbonyl, alkoxycarbonyl groups as defined herein.

The term "SMIP compound" refers to small molecule immunopotentiating compounds, that include small molecule compounds below about MW 1000 g/mol, preferably MW 800 g/mol that are capable of stimulating or modulating a pro-inflammatory response in a patient. In an embodiment, the SMIP compounds are able to stimulate human peripheral blood mononuclear cells to produce cytokines. Preferred SMIP compounds and derivatives thereof include, for example, aminoazavinyl compounds, benzazole compounds, acylpiperazine compounds, indoledione compounds, tetrahydroisoquinoline (THIQ) compounds, anthraquinone compounds, indanedione compounds, pthalimide compounds, benzocyclodione compounds, aminobenzimidazole quinolinone (ABIQ) compounds, hydraphthalimide compounds, pyrazolopyrimidine compounds, quinazilinone compounds, quinoxaline compounds, triazine compounds, tetrahydropyrrolidinoquinoxaline compounds, pyrrole compounds, benzophenone compounds, sterol compound, and isoxazole compounds.

The term "SMIS compound" refers to small molecule immunosuppressant compounds, that include small molecule compounds below about about MW 1000 g/mol, preferably MW 800 g/mol, capable of suppressing or modulating a pro-inflammatory response in a patient.

Acylpiperazine compounds as described throughout this application include compounds of formula (III) as shown below:



III

wherein,

R_9 is selected from the group consisting of substituted or unsubstituted aryl, heteroaryl, arylalkyl, arylalkenyl, heteroarylalkyl, and heteroarylalkenyl;

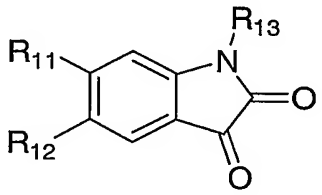
R_{10} is substituted or unsubstituted alkyl;

n is an integer from 0-2; and

if D_1 is carbon than D_2 is oxygen, D_3 is absent, and D_4 is selected from the group consisting of substituted or unsubstituted aryl, heteroaryl, carbocycyl, alkoxyaryl, fused arylaryl, fused arylheteroaryl, and fused heteroarylaryl; or,

if D_1 is nitrogen than D_2 is nitrogen, D_4 is absent, and D_3 is selected from the group consisting of substituted or unsubstituted aryl, heteroaryl, carbocycyl, alkoxyaryl, fused arylaryl, fused arylheteroaryl, and fused heteroarylaryl.

Indoledione compounds as described throughout this application include compounds of formula (IV) as shown below:



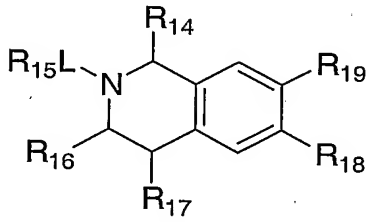
IV

wherein,

R_{11} and R_{12} are independently selected from the group consisting of H, nitro, halogen, amino, hydroxy, cyano, carboxcyclic acid, and substituted or unsubstituted alkyl, aryl, heteroaryl, alkoxy, alkylcarbonyl, alkylcarbonylamino, alkylaminocarbonyl, aminocarbonyl, arylalkoxy, heteroarylalkoxy, alkylamino, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, and carbocyclyl groups; and,

R_{13} is selected from the group consisting of substituted or unsubstituted aryl, heteroaryl, arylalkyl, heteroarylalkyl, heterocyclyl, heterocyclylalkyl, and alkylbenzyl.

Tetrahydroisoquinoline (THIQ) compounds as described throughout this application include compounds of formula (V) as shown below:



V

wherein,

L is a covalent bond or selected from the group consisting of $-CH_2-$, -CO-, -O-, -S-, CHF, -NH-, $-NR_{20}-$, where R_{20} is lower alkyl;

R_{14} is selected from the group consisting of hydrogen, halogen, and substituted or unsubstituted alkyl;

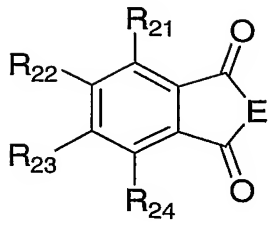
R_{15} is selected from the group consisting of substituted or unsubstituted carbocyclyl, aryl, arylalkyl, alkoxyaryl, heteroaryl, heterocyclyl;

R_{16} is selected from the group consisting of hydrogen, halogen, and substituted or unsubstituted alkyl;

R_{17} is selected from the group consisting of hydrogen, halogen, and substituted or unsubstituted alkyl; and,

R_{18} and R_{19} are independently selected from the group consisting of H, hydroxy, halogen, alkoxy, amino, unsubstituted alkyl, substituted alkyl, and alkylamino.

Benzocyclodione compounds as described throughout this application include compounds of formula (VI) as shown below:



VI

wherein,

E is selected from the group consisting of NR_{25} or $CR_{26}R_{27}$;

R_{21}, R_{23}, and R_{24} are independently selected from the group consisting of H, hydroxy, halogen, alkoxy, amino, unsubstituted alkyl, substituted alkyl, and alkylamino;

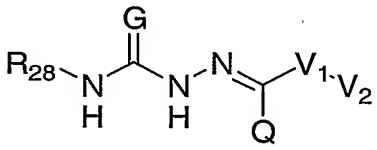
R_{22} is selected from the group consisting or H, hydroxy, halogen, alkoxy, amino, and unsubstituted or substituted alkyl, and alkylamino, arylalkyl, heteroarylalkyl, aryl, heteroaryl, arylcarbonyl, heterocyclyl, heterocyclylalkyl, and heteroarylcarbonyl;

R_{25} is selected from the group consisting of substituted or unsubstituted aryl, heteroaryl, heterocyclyl, carbocyclyl, arylalkyl, heteroarylalkyl, and heterocyclyalkyl;

R_{26} is selected from the group consisting of H, halogen, hydroxy, amino, and substituted or unsubstituted alkyl, carbonylalkyl, and alkylcarbonylalkyl; and,

R_{27} is selected from the group aryl, arylalkyl, heteroarylalkyl, heterocyclyl, heterocyclylalkyl, carbocyclyl, arylcarbonylalkyl, and arylalkylcarbonyl.

Aminoazavinyl compounds as described throughout this application include compounds of formula (VII) as shown below:



VII

wherein,

G is either S or NH;

R_{28} is selected from the group consisting of H, and substituted or unsubstituted alkyl, aryl, heteroaryl, heteroarylalkyl, arylalkyl, carbocyclyl, carbocyclylalkyl, heterocyclyl, and heterocyclylalkyl;

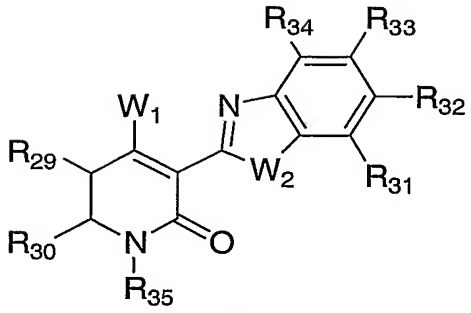
Q is selected from the group consisting of hydrogen, substituted alkyl, unsubstituted alkyl, and aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclyl, substituted

heterocyclyl, fused or unfused arylaryl, substituted arylaryl, arylheteroaryl, substituted arylheteroaryl, heteroarylheteroaryl, and substituted heteroarylheteroaryl;

V_1 is selected from the group consisting of alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, alkoxy, substituted alkoxy, aminocarbonyl, alkoxycarbonyl, carboxyl sulfonyl, methanesulfonyl, and substituted or unsubstituted alkylcarbonyl, arylcarbonyl, aralkylcarbonyl, heteroarylcarbonyl, heteroaralkylcarbonyl, alkylcarbonyloxy, arylcarbonyloxy, aralkylcarbonyloxy, heteroarylcarbonyloxy, heteroaralkylcarbonyloxy, alkylaminocarbonyloxy, arylaminocarbonyloxy, formyl, loweralkylcarbonyl, loweralkoxycarbonyl, aminocarbonyl, aminoaryl, alkylsulfonyl, sulfonamido, aminoalkoxy, alkylamino, heteroarylamino, alkylcarbonylamino, alkylaminocarbonylamino, arylaminocarbonylamino, aralkylcarbonylamino, heteroarylcarbonylamino, arylcarbonylamino, cycloamidino, cycloalkyl, cycloimido, arylsulfonyl and arylsulfonamido; and,

V_2 is selected from the group consisting of hydrodgen, halogen, alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, alkoxy, substituted alkoxy, aminocarbonyl, alkoxycarbonyl, carboxyl sulfonyl, methanesulfonyl, and substituted or unsubstituted alkylcarbonyl, arylcarbonyl, aralkylcarbonyl, heteroarylcarbonyl, heteroaralkylcarbonyl, alkylcarbonyloxy, arylcarbonyloxy, aralkylcarbonyloxy, heteroarylcarbonyloxy, heteroaralkylcarbonyloxy, alkylaminocarbonyloxy, arylaminocarbonyloxy, formyl, loweralkylcarbonyl, loweralkoxycarbonyl, aminocarbonyl, aminoaryl, alkylsulfonyl, sulfonamido, aminoalkoxy, alkylamino, heteroarylamino, alkylcarbonylamino, alkylaminocarbonylamino, arylaminocarbonylamino, aralkylcarbonylamino, heteroarylcarbonylamino, arylcarbonylamino, cycloamidino, cycloalkyl, cycloimido, arylsulfonyl and arylsulfonamido.

Lactam compounds as described throughout this application include compounds of formula (VIII) as shown below:



VIII

wherein,

W_1 is selected from the group consisting of –OH, -OR_{36} groups, -$NR_{37}R_{38}$;

W2 is selected from the group consisting of O, S, and NR_{39} groups;

R_{29} and R_{30} join to form a 5 to 6 membered substituted or unsubstituted ring comprising all carbon atoms or at least one O, N, or S atom;

R_{35} and R_{39} may be the same or different and are selected from the group consisting of H, -OH substituted and unsubstituted alkyl groups, substituted and unsubstituted aryl groups, -C(=O)H, -C(=O)-alkyl groups, and –C(=O)-aryl groups;

R_{31}, R_{32}, R_{33}, and R_{34} may be the same or different and are independently selected from the group consisting of H, Cl, Br, F, I, -NO_2, -CN, -OH, -OR_{40} groups, -$NR_{41}R_{42}$ groups, -C(=O)R_{43} groups, -SH groups, substituted and unsubstituted amidinyl groups, substituted and unsubstituted guanidinyl groups, substituted and unsubstituted alkyl groups, substituted and unsubstituted aryl groups, substituted and unsubstituted alkenyl groups, substituted and unsubstituted alkynyl groups, substituted and unsubstituted heterocyclyl groups, substituted and unsubstituted alkylaminoalkyl groups, substituted and unsubstituted dialkylaminoalkyl groups, substituted and unsubstituted arylaminoalkyl groups, substituted and unsubstituted diarylaminoalkyl groups, substituted and unsubstituted (alkyl)(aryl)aminoalkyl groups, substituted and unsubstituted heterocyclylalkyl groups, substituted and unsubstituted aminoalkyl groups, substituted and unsubstituted heterocyclylaminoalkyl groups, substituted and unsubstituted diheterocyclylaminoalkyl groups, substituted and unsubstituted (alkyl)(heterocyclyl)aminoalkyl groups, substituted and unsubstituted (aryl)(heterocyclyl)aminoalkyl groups, substituted and unsubstituted hydroxyalkyl groups, substituted and unsubstituted alkoxyalkyl groups, substituted and unsubstituted aryloxyalkyl groups, and substituted and unsubstituted heterocyclyloxyalkyl groups;

R_{36} is selected from the group consisting of substituted and unsubstituted alkyl groups, substituted and unsubstituted aryl groups, substituted and unsubstituted heterocyclyl

groups, substituted and unsubstituted heterocyclylalkyl groups, -C(=O)H, -C(=O)-alkyl groups, –C(=O)-aryl groups, -C(=O)O-alkyl groups, -C(=O)O-aryl groups, -C(=O)NH_2, -C(=O)NH(alkyl) groups, -C(=O)NH(aryl) groups, -C(=O)N(alkyl)$_2$ groups, -C(=O)N(aryl)$_2$ groups, -C(=O)N(alkyl)(aryl) groups, -NH_2, -NH(alkyl) groups, -NH(aryl) groups, -N(alkyl)$_2$ groups, -N(alkyl)(aryl) groups, -N(aryl)$_2$ groups, -C(=O)NH(heterocyclyl) groups, -C(=O)N(heterocyclyl)$_2$ groups, -C(=O)N(alkyl)(heterocyclyl) groups, and -C(=O)N(aryl)(heterocyclyl) groups;

R_{37} is selected from the group consisting of H, substituted and unsubstituted alkyl groups, substituted and unsubstituted aryl groups, and substituted and unsubstituted heterocyclyl groups;

R_{38} is selected from the group consisting of H, substituted and unsubstituted alkyl groups, substituted and unsubstituted aryl groups, substituted and unsubstituted heterocyclyl groups, -OH, alkoxy groups, aryloxy groups, -NH_2, substituted and unsubstituted heterocyclylalkyl groups, substituted and unsubstituted aminoalkyl groups, substituted and unsubstituted alkylaminoalkyl groups, substituted and unsubstituted dialkylaminoalkyl groups, substituted and unsubstituted arylaminoalkyl groups, substituted and unsubstituted diarylaminoalkyl groups, substituted and unsubstituted (alkyl)(aryl)aminoalkyl groups, substituted and unsubstituted alkylamino groups, substituted and unsubstituted arylamino groups, substituted and unsubstituted dialkylamino groups, substituted and unsubstituted diarylamino groups, substituted and unsubstituted (alkyl)(aryl)amino groups, -C(=O)H, -C(=O)-alkyl groups, -C(=O)-aryl groups, -C(=O)O-alkyl groups, -C(=O)O-aryl groups, -C(=O)NH_2, -C(=O)NH(alkyl) groups, -C(=O)NH(aryl) groups, -C(=O)N(alkyl)$_2$ groups, -C(=O)N(aryl)$_2$ groups, -C(=O)N(alkyl)(aryl) groups, -C(=O)-heterocyclyl groups, -C(=O)-O-heterocyclyl groups, -C(=O)NH(heterocyclyl) groups, -C(=O)-N(heterocyclyl)$_2$ groups, -C(=O)-N(alkyl)(heterocyclyl) groups, -C(=O)-N(aryl)(heterocyclyl) groups, substituted and unsubstituted heterocyclylaminoalkyl groups, substituted and unsubstituted diheterocyclylaminoalkyl groups, substituted and unsubstituted (alkyl)(heterocyclyl)aminoalkyl groups, substituted and unsubstituted (aryl)(heterocyclyl)aminoalkyl groups, substituted and unsubstituted hydroxyalkyl groups, substituted and unsubstituted alkoxyalkyl groups, substituted and unsubstituted aryloxyalkyl groups, and substituted and unsubstituted heterocyclyloxyalkyl groups;

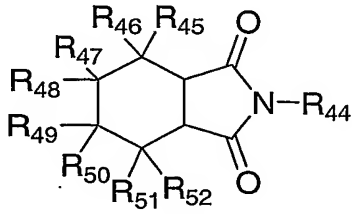
R_{41} is selected from the group consisting of H, substituted and unsubstituted alkyl groups, substituted and unsubstituted aryl groups, and substituted and unsubstituted heterocyclyl groups;

R_{42} is selected from the group consisting of H, substituted and unsubstituted alkyl groups, substituted and unsubstituted aryl groups, substituted and unsubstituted heterocyclyl groups, -C(=O)H, -C(=O)-alkyl groups, –C(=O)-aryl groups, -$C(=O)NH_2$, -C(=O)NH(alkyl) groups, -C(=O)NH(aryl) groups, -$C(=O)N(alkyl)_2$ groups, -$C(=O)N(aryl)_2$ groups, -C(=O)N(alkyl)(aryl) groups, -C(=O)O-alkyl groups, -C(=O)O-aryl groups, substituted and unsubstituted aminoalkyl groups, substituted and unsubstituted alkylaminoalkyl groups, substituted and unsubstituted dialkylaminoalkyl groups, substituted and unsubstituted arylaminoalkyl groups, substituted and unsubstituted diarylaminoalkyl groups, substituted and unsubstituted (alkyl)(aryl)aminoalkyl groups, substituted and unsubstituted heterocyclylalkyl groups, -C(=O)-heterocyclyl groups, -C(=O)-O-heterocyclyl groups, -C(=O)NH(heterocyclyl) groups, -C(=O)-$N(heterocyclyl)_2$ groups, -C(=O)-N(alkyl)(heterocyclyl) groups, -C(=O)-N(aryl)(heterocyclyl) groups, substituted and unsubstituted heterocyclylaminoalkyl groups, substituted and unsubstituted diheterocyclylaminoalkyl groups, substituted and unsubstituted (heterocyclyl)(alkyl)aminoalkyl groups, substituted and unsubstituted (heterocyclyl)(aryl)aminoalkyl groups, substituted and unsubstituted hydroxyalkyl groups, substituted and unsubstituted alkoxyalkyl groups, substituted and unsubstituted aryloxyalkyl groups, and substituted and unsubstituted heterocyclyloxyalkyl groups; and

R_{43} is selected from the group consisting of H, -NH_2, -NH(alkyl) groups, -NH(aryl) groups, -$N(alkyl)_2$ groups, -$N(aryl)_2$ groups, -N(alkyl)(aryl) groups, -NH(heterocyclyl) groups, -N(heterocyclyl)(alkyl) groups, -N(heterocyclyl)(aryl) groups, -$N(heterocyclyl)_2$ groups, substituted and unsubstituted alkyl groups, substituted and unsubstituted aryl groups, -OH, substituted and unsubstituted alkoxy groups, substituted and unsubstituted heterocyclyl groups, substituted and unsubstituted aryloxy groups, heterocyclyloxy groups, -NHOH, -N(alkyl)OH groups, -N(aryl)OH groups, -N(alkyl)O-alkyl groups, -N(aryl)O-alkyl groups, -N(alkyl)O-aryl groups, and -N(aryl)O-aryl groups.

Preferably R_{29} and R_{30} join together to form a substituted or unsubstituted phenyl ring.

Hydropthalamide compounds as described throughout this application include compounds of formula (IX) as shown below:



IX

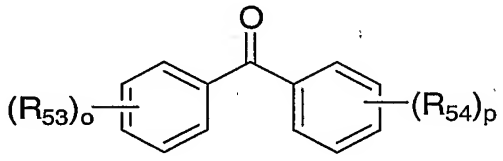
wherein,

R_{44} is selected from the group consisting of substituted or unsubstituted aryl, heteroaryl, arylalkyl, heteroarylalkyl, fused arylaryl, unfused arylaryl, fused heteroarylaryl, unfused heteroarylaryl, fused arylheteroaryl, and unfused arylheteroaryl;

R_{45}, R_{47}, R_{49}, and R_{51} may be the same or different and are independently selected from the group consisting of H, nitro, halogen, amino, hydroxy, cyano, carboxcyclic acid, and substituted or unsubstituted alkyl, aryl, heteroaryl, alkoxy, alkylcarbonyl, alkylcarbonylamino, alkylaminocarbonyl, aminocarbonyl, arylalkoxy, heteroarylalkoxy, alkylamino, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, and carbocyclyl; and

R_{46}, R_{48}, R_{50}, and R_{52} may be the same or different and are independently selected from the group consisting of H, halogen, and substituted or unsubstituted alkyl groups.

Benzophenone compounds as described throughout this application include compounds of formula (X) as shown below:



X

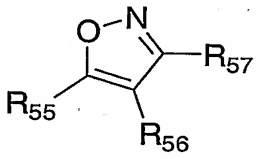
wherein,

R_{53} is independently selected from the group consisting of H, nitro, halogen, amino, hydroxy, cyano, carboxcyclic acid, and substituted or unsubstituted alkyl, aryl, heteroaryl, alkoxy, alkylcarbonyl, alkylcarbonylamino, alkylaminocarbonyl, aminocarbonyl, arylalkoxy, heteroarylalkoxy, alkylamino, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, and carbocyclyl;

R_{54} is independently selected from the group consisting of H, nitro, halogen, amino, hydroxy, cyano, carboxcyclic acid, and substituted or unsubstituted alkyl, aryl, heteroaryl, alkoxy, alkylcarbonyl, alkylcarbonylamino, alkylaminocarbonyl, aminocarbonyl, arylalkoxy, heteroarylalkoxy, alkylamino, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, and carbocyclyl; and

o and p are integers from 0-4.

Isoxazole compounds as described throughout this application include compounds of formula (XI) as shown below:



XI

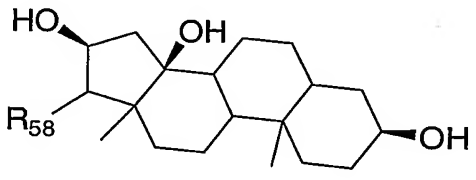
wherein,

R_{55} is selected from the group consisting of substituted or unsubstituted aryl, arylalkyl, heteroaryl, heteroarylalkyl, heterocyclyl, and heterocyclylalkyl;

R_{56} is selected from the group consisting of substituted or unsubstituted aryl, arylalkyl, heteroaryl, heteroarylalkyl, heterocyclyl, and heterocyclylalkyl; and,

R_{57} is selected from the group consisting of H, halogen, hydoxy, and substituted or unsubstituted alkyl, aryl, heteroaryl, heterocyclyl, and carbonyl.

Sterol compounds as described throughout this application include compounds of formula (XII) as shown below:



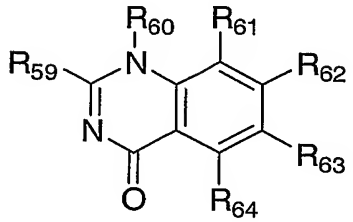
XII

wherein,

R_{58} is selected from the group consisting of substituted or unsubstituted aryl, arylalkyl, heteroaryl, heteroarylalkyl, heterocyclyl, and heterocyclylalkyl.

Preferably R_{58} is a pyranone substituent.

Quinazilinone compounds as described throughout this application include compounds of formula (XIII) as shown below:



XIII

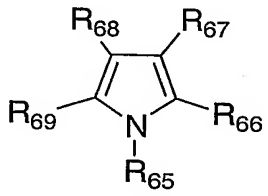
wherein,

R_{59} is selected from the group consisting of H, halogen, hydroxy, and substituted or unsubstituted alkyl, aminoalkyl, alklyaminoalkyl, alkoxy, dialkylaminoalkyl, hydroxyalkyl, alkenyl, alkynyl, carbocyclyl, carbocyclylalkyl, heterocyclyl, and heterocyclylalkyl;

R_{60} is selected from the group consisting of substituted or unsubstituted aryl, heteroaryl, arylalkyl, heteroarylalkyl, and heterocyclylalkyl; and,

R_{61}, R_{62}, R_{63}, and R_{64} may be the same or different and are independently selected from the group consisting of H, nitro, halogen, amino, hydroxy, cyano, carboxcyclic acid, and substituted or unsubstituted alkyl, aryl, heteroaryl, alkoxy, alkylcarbonyl, alkylcarbonylamino, alkylaminocarbonyl, aminocarbonyl, arylalkoxy, heteroarylalkoxy, alkylamino, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, and carbocyclyl groups.

Pyrrole compounds as described throughout this application include compounds of formula (XIV) as shown below:



XIV

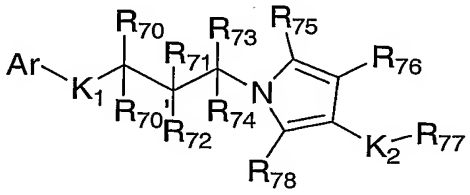
wherein,

R_{65} is selected from the group consisting of H, hydroxy, and substituted or unsubstituted alkyl, aryl, heteroaryl, heteroarylalkyl, arylalkyl, heteroarylaminoalkyl, arylaminoalkyl, heteroaryloxyalkyl, and aryloxyalkyl groups;

R_{66}, R_{67}, R_{68}, and R_{69} may be the same or different and are independently selected from the group consisting of H, nitro, halogen, amino, hydroxy, cyano, carboxcyclic acid, and

substituted or unsubstituted alkyl, aryl, heteroaryl, alkoxy, alkylcarbonyl, alkylcarbonylamino, alkylaminocarbonyl, aminocarbonyl, arylalkoxy, heteroarylalkoxy, alkylamino, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, and carbocyclyl groups.

Further preferred pyrrole compounds include those shown in Formula (XV):



(XV)

wherein:

K_1 is nitrogen, oxygen, or optionally substituted carbon;

W is absent or is selected from the group consisting of –O-, -S-, -S(O)-, -SO_2-, -NH-, -NH-CO-, -NR'CO-, -$NHSO_2$-, -NR'SO_2-, -CO-, -CO_2--, -CH_2--, -CF_2--, CHF, -CONH-, -CONR'-, and –NR'-, where R' is alkyl, substituted alkyl, cycloalkyl, aryl, heteroaryl, heterocyclo;

Ar is optionally substituted aryl, heteroaryl, or a protecting group;

R_{70} and R_{70}' are independently selected from the group consisting of hydrogen and methyl;

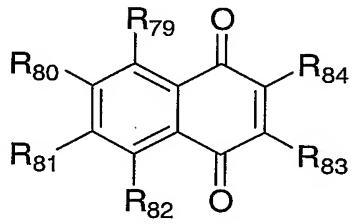
R_{71}, R_{72}, R_{73}, and R_{74} are independently selected from the group consisting of hydrogen, hydroxyl, and optionally substituted loweralkyl, cyclooweralkyl, cyclicaminoalkyl, alkylaminoalkyl, loweralkoxy, amino, alkylamino, alkylcarbonyl, arylcarbonyl, aralkylcarbonyl, heteroarylcarbonyl, heteroaralkylcarbonyl, aryl and heteroaryl;

R_{75} and R_{78} are independently selected from the group consisting of hydrogen, halo, and optionally substituted loweralkyl, cycloalkyl, alkoxy, amino, aminoalkoxy, carbonyloxy, aminocarbonyloxy, alkylcarbonylamino, arylcarbonylamino, aralkylcarbonylamino, heteroarylcarbonylamino, heteroaralkylcarbonylamino, cycloimido, heterocycloimido, amidino, cycloamidino, heterocycloamidino, guanidinyl, aryl, heteroaryl, heterocycloalkyl, heterocyclocarbonyloxy, heteroarylcarbonyloxy, and arylsulfonamido;

R_{76} is selected from the group consisting of hydrogen, aryl, heteroaryl, substituted heteroaryl, heterocyclyl, and substituted heterocyclyl;

R_{77} is selected from the group consisting of hydrogen, hydroxy, halo, carboxyl, nitro, amino, amido, amidino, imido, cyano, sulfonyl, methanesulonyl, and substituted or unsubstituted alkyl, alkoxy, alkylcarbonyl, arylcarbonyl, aralkylcarbonyl, heteroarylcarbonyl, heteroaralkylcarbonyl, alkylcarbonyloxy, arylcarbonyloxy, aralkylcarbonyloxy, heteroarylcarbonyloxy, heteroaralkylcarbonyloxy, alkylaminocarbonyloxy, arylaminocarbonyloxy, formyl, loweralkylcarbonyl, loweralkoxycarbonyl, aminocarbonyl, aminoaryl, alkylsulfonyl, sulfonamido, aminoalkoxy, alkylamino, heteroarylamino, alkylcarbonylamino, alkylaminocarbonylamino, arylaminocarbonylamino, aralkylcarbonylamino, heteroarylcarbonylamino, arylcarbonylamino, heteroarylcarbonylamino cycloamido, cyclothioamido, cycloamidino, heterocycloamidino, cycloalkyl, cycloimido, heterocycloimido, guanidinyl, aryl, heteroaryl, heterocyclo, heterocycloalkyl, arylsulfonyl and arylsulfonamido;

Anthraquinone compounds of the instant invention include, for example, compounds of Formula (XVI):



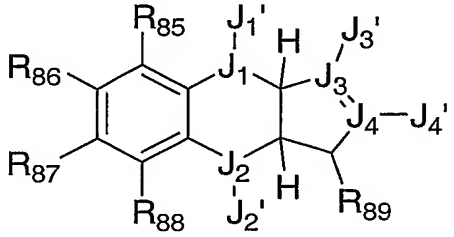
XVI

wherein,

R_{79}, R_{80}, R_{81}, and R_{82} may be the same or different and are independently selected from the group consisting of H, nitro, halogen, amino, hydroxy, cyano, carboxcyclic acid, and substituted or unsubstituted alkyl, aryl, heteroaryl, alkoxy, alkylcarbonyl, alkylcarbonylamino, sulfonyl, aminosulfonyl, alkylaminocarbonyl, aminocarbonyl, arylalkoxy, heteroarylalkoxy, alkylamino, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, and carbocyclyl groups; and,

R_{83} and R_{84} are taken together to form a substituted or unsubstituted 5-6 membered ring containing all carbon atoms or 1-2 heteroatoms selected from the group consisting of O, S, and N.

Quinoxaline compounds referred to throughout this application include tricyclic, partially unconjugated compounds optionally substituted with nitrogen heteroatoms as shown in the preferred quinoxaline embodiment (XVII) below:



XVII

wherein,

J_1 is either C or N,

J_1' is selected from the group consisting of H, substituted aryl, unsubstituted aryl, substituted heteroaryl, and unsubstituted heteroaryl;

J_2 is either C or N,

J_2' is selected from the group consisting of H, substituted aryl, unsubstituted aryl, substituted heteroaryl, and unsubstituted heteroaryl;

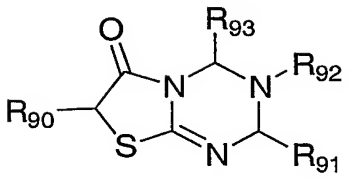
J_3 is selected from the group consisting of -CO-, -NH-, and -N=;

if J_4 is -O- then J_4' is absent; or,

if J_4 is =C- then J_4' is selected from the group consisting of H and substituted or unsubstituted alkyl, alkoxy, aryl, heteroaryl, heteroarylalkyl, arylalkyl, aminoalkyl, alkylamino, and alkylthio groups; and,

R_{85}, R_{86}, R_{87}, R_{88}, and R_{89} may be the same or different and are independently selected from the group consisting of H, nitro, halogen, amino, hydroxy, cyano, carboxcyclic acid, and substituted or unsubstituted alkyl, aryl, heteroaryl, alkoxy, alkylcarbonyl, alkylcarbonylamino, sulfonyl, aminosulfonyl, alkylaminocarbonyl, aminocarbonyl, arylalkoxy, heteroarylalkoxy, alkylamino, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, and carbocyclyl groups.

Triazine compounds refer to substituted 6-membered heterocyclic groups with 3 nitrogen atoms distributed throughout the ring. The preferred embodiments of the instant invention include those shown in structures (XVIII), (XIX) and (XX) shown below:



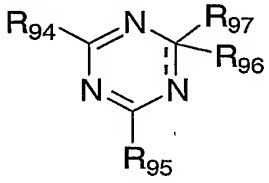
XVIII

wherein,

R_{90} is selected from the group consisting of substituted or unsubstituted alkyl, alkenyl, akynyl, aryl, heteroaryl, heteroarylalkyl, heteroarylalkenyl, arylalkyl, and arylalkenyl;

R_{91} and R_{93} are independently selected from the group consisting of H, and unsubstituted alkyl;

R_{91} is aryl; preferably phenyl,



XIX

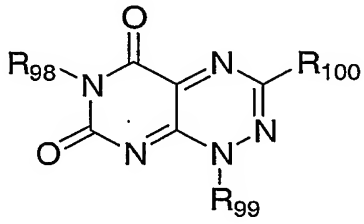
wherein,

R_{94} is selected from the group consisting of H, amino, alkyl, aminoalkyl, and halogen;

R_{95} is selected from the group consisting of substituted or unsubstituted aryl, arylamino, arylalkylamino, heteroaryl, heteroarylamino, and heteroalkylamino;

R_{96} and R_{97} are independently selected from the group consisting of H, halogen, and alkyl, preferably methyl; or,

R_{96} may form a double bond with the nitrogen atom directly below it as indicated by the dashed line in the above structure; and,



XX

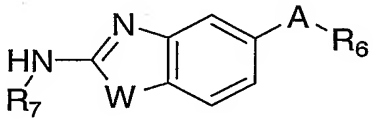
wherein,

R_{98} is selected from the group consisting of H, substituted alkyl, and unsubstituted alkyl; preferably methyl,

R_{99} is selected from the group consisting of H, substituted alkyl, and unsubstituted alkyl; preferably ethyl,

R_{100} is selected from the group consisting of substituted or unsubstituted aryl, heteroaryl, alkoxyaryl, arylalkyl, and heteroarylalkyl.

Benzazole compounds as described throughout this application include compounds of formula (XXI) as shown below:



XXI

wherein,

A is selected from the group consisting of -O-, -S-, -NH-, and $-NR_8-$;

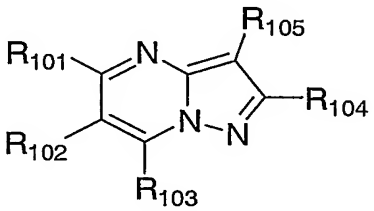
W is selected from the group consisting of $-CH_2-$, -O-, -S-, -NH-, and $-NR_8-$;

R_7 is selected from the group consisting of carbocyclyl, unfused carbocyclylcarbocyclyl, substituted aryl, unsubstituted aryl, substituted heteroaryl, unsubstituted heteroaryl, substituted fused arylheteroaryl, unsubstituted fused arylheteroaryl, substituted unfused arylaryl and unsubstituted unfused arylaryl;

R_6 is selected from the group consisting of substituted or unsubstituted aryl, and heteroaryl; and,

R_8 is independently substituted or unsubstituted alkyl.

Pyrazalopyrimidine compounds as described throughout this application include compounds of formula (XXII) as shown below:



XXII

wherein,

R_{101} is selected from the group consisting of H, nitro, halogen, amino, hydroxy, cyano, carboxcyclic acid, and substituted or unsubstituted alkyl, aryl, heteroaryl, alkoxy, alkylcarbonyl, alkylcarbonylamino, sulfonyl, aminosulfonyl, alkylaminocarbonyl, aminocarbonyl, arylalkoxy, heteroarylalkoxy, alkylamino, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, and carbocyclyl groups;

R_{102} is selected from the group consisting of H, nitro, halogen, amino, hydroxy, cyano, carboxcyclic acid, and substituted or unsubstituted alkyl, aryl, heteroaryl, alkoxy, alkylcarbonyl, alkylcarbonylamino, alkylaminocarbonyl, aminocarbonyl, arylalkoxy, heteroarylalkoxy, alkylamino, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, and carbocyclyl groups;

R_{103} is selected from the group consisting of H, nitro, halogen, amino, hydroxy, cyano, carboxcyclic acid, trifluoromethyl, and substituted or unsubstituted alkyl, aryl, heteroaryl, alkoxy, alkylcarbonyl, alkylcarbonylamino, alkylaminocarbonyl, aminocarbonyl, arylalkoxy, heteroarylalkoxy, alkylamino, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, and carbocyclyl groups;

R_{104} is selected from the group consisting of H and substituted or unsubstituted aryl, heteroaryl, arylalkoxy, heteroarylalkoxy, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, carbocyclylalkyl and carbocyclyl groups;

R_{105} is selected from the group consisting of H and substituted or unsubstituted aryl, heteroaryl, arylalkoxy, heteroarylalkoxy, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, carbocyclylalkyl and carbocyclyl groups;

wherein at least one of R_{104} and R_{105} is not H.

SMIP compounds identified by *in-vitro* (cellular or non-cellular assays) or *in-vivo* methods are thoroughly described in Methods 1 and 2 below.

Pharmaceutical compositions containing the compounds of the invention may be in any form suitable for the intended method of administration, including, for example, a solution, a suspension, or an emulsion. Liquid carriers are typically used in preparing solutions, suspensions, and emulsions. Liquid carriers contemplated for use in the practice of the present invention include, for example, water, saline, pharmaceutically acceptable organic solvent(s), pharmaceutically acceptable oils or fats, and the like, as well as mixtures of two or more thereof. The liquid carrier may contain other suitable pharmaceutically acceptable additives such as solubilizers, emulsifiers, nutrients, buffers, preservatives, suspending agents, thickening agents, viscosity regulators, stabilizers, and the like. Suitable organic solvents include, for example, monohydric alcohols, such as ethanol, and polyhydric alcohols, such as glycols. Suitable oils include, for example, soybean oil, coconut oil, olive oil, safflower oil, cottonseed oil, and the like. For parenteral administration, the carrier can also be an oily ester such as ethyl oleate, isopropyl myristate, and the like. Compositions of the present invention may also be in the form of microparticles, microcapsules, liposomal encapsulates, and the like, as well as combinations of any two or more thereof.

Other additives include immunostimulatory agents known in the art. Immunostimulatory oligonucleotides and polynucleotides are described in PCT WO 98/55495 and PCT WO 98/16247. U.S. Patent Application No. 2002/0164341 describes adjuvants including an unmethylated CpG dinucleotide (CpG ODN) and a non-nucleic acid adjuvant. U.S. Patent Application No. 2002/0197269 describes compositions comprising an antigen, an antigenic CpG-ODN and a polycationic polymer. Other immunostimulatory additives described in the art may be used, for example, as described in U.S. Patent No. 5,026,546; U.S. Patent No. 4,806,352; and U.S. Patent No. 5,026,543.

A controlled release delivery system may be used, such as a diffusion controlled matrix system or an erodible system, as described for example in: Lee, "Diffusion-Controlled Matrix Systems", pp. 155-198 and Ron and Langer, "Erodible Systems", pp. 199-224, in "Treatise on Controlled Drug Delivery", A. Kydonieus Ed., Marcel Dekker, Inc., New York 1992. The matrix may be, for example, a biodegradable material that can degrade spontaneously *in situ* and *in vivo* for, example, by hydrolysis or enzymatic cleavage, *e.g.*, by proteases. The delivery system may be, for example, a naturally occurring or synthetic polymer or copolymer, for example in the form of a hydrogel. Exemplary polymers with cleavable linkages include polyesters, polyorthoesters, polyanhydrides, polysaccharides, poly(phosphoesters), polyamides, polyurethanes, poly(imidocarbonates) and poly(phosphazenes).

The compounds of the invention may be administered enterally, orally, parenterally, sublingually, by inhalation spray, rectally, or topically in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles as desired. For example, suitable modes of administration include oral, subcutaneous, transdermal, transmucosal, iontophoretic, intravenous, intramuscular, intraperitoneal, intranasal, subdermal, rectal, and the like. Topical administration may also involve the use of transdermal administration such as transdermal patches or ionophoresis devices. The term parenteral includes subcutaneous injections, intravenous, intramuscular, intrasternal injection, or infusion techniques.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-propanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Suppositories for rectal administration of the drug can be prepared by mixing the drug with a suitable nonirritating excipient such as cocoa butter and polyethylene glycols that are solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum and release the drug.

Solid dosage forms for oral administration may include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound may be admixed with at least one inert diluent such as sucrose lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration may include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions may also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, cyclodextrins, and sweetening, flavoring, and perfuming agents.

As to the mode of administration, it should be emphasized that it is the combination of therapeutic agents that gives rise to its synergistic therapeutic effect no matter whether the first and the second agent are administered together or separately. Therefore, the two agents may be given together in a single dose or in separate ones with respect to space and time.

Effective amounts of the compounds of the invention generally include any amount sufficient to detectably treat viral infections.

Successful treatment of a subject in accordance with the invention may result in the inducement of a reduction or alleviation of symptoms in a subject afflicted with a medical or biological disorder to, for example, halt the further progression of the disorder, or the prevention of the disorder.

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination, and the severity of the particular disease undergoing therapy. The therapeutically effective amount for a given situation can be readily determined by routine experimentation and is within the skill and judgment of the ordinary clinician.

The compounds of the present invention can also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multilamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain, in addition to a compound of the present invention, stabilizers, preservatives, excipients, and the like. The preferred lipids are the phospholipids and phosphatidyl cholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art. See, for example, Prescott, Ed., *Methods in Cell Biology*, Volume XIV, Academic Press, New York, N.W., p. 33 *et seq* (1976).

While the SMIP compounds of the invention can be administered as the sole active pharmaceutical agent, they can also be used in combination with one or more other agents used in the treatment of SARSs. Other representative agents useful in combination with the

compounds of the invention for the treatment of viral infections include, for example, interferon, ribavirin, gancyclovir and the like.

When additional active agents are used in combination with the compounds of the present invention, the additional active agents may generally be employed in therapeutic amounts as indicated in the PHYSICIANS' DESK REFERENCE (PDR) 53rd Edition (1999), that is incorporated herein by reference, or such therapeutically useful amounts as would be known to one of ordinary skill in the art.

The compounds of the invention and the other therapeutically active agents can be administered at the recommended maximum clinical dosage or at lower doses. Dosage levels of the active compounds in the compositions of the invention may be varied so as to obtain a desired therapeutic response depending on the route of administration, severity of the disease and the response of the patient. The combination can be administered as separate compositions or as a single dosage form containing both agents. When administered as a combination, the therapeutic agents can be formulated as separate compositions that are given at the same time or different times, or the therapeutic agents can be given as a single composition.

Compounds of the present invention can be readily synthesized using the methods described herein, or other methods, that are well known in the art.

The compounds can be used in the form of salts derived from inorganic or organic acids. These salts include but are not limited to the following: acetate, adipate, alginate, citrate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, camphorate, camphorsulfonate, digluconate, cyclopentanepropionate, dodecylsulfate, ethanesulfonate, glucoheptanoate; glycerophosphate, hemisulfate, heptanoate, hexanoate, fumarate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, nicotinate, 2-napthalenesulfonate, oxalate, pamoate, pectinate, persulfate, 3-phenylproionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, p-toluenesulfonate and undecanoate. Also, the basic nitrogen-containing groups can be quaternized with such agents as loweralkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides, and iodides; dialkyl sulfates like dimethyl, diethyl, dibutyl, and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides like benzyl and phenethyl bromides, and others. Water or oil-soluble or dispersible products are thereby obtained.

Examples of acids that may be employed to form pharmaceutically acceptable acid addition salts include such inorganic acids as hydrochloric acid, sulphuric acid and phosphoric acid and such organic acids as oxalic acid, maleic acid, succinic acid and citric acid. Basic

addition salts can be prepared *in situ* during the final isolation and purification of the compounds of formula (I), or separately by reacting carboxylic acid moieties with a suitable base such as the hydroxide, carbonate or bicarbonate of a pharmaceutical acceptable metal cation or with ammonia, or an organic primary, secondary or tertiary amine. Pharmaceutical acceptable salts include, but are not limited to, cations based on the alkali and alkaline earth metals, such as sodium, lithium, potassium, calcium, magnesium, aluminum salts and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. Other representative organic amines useful for the formation of base addition salts include diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like.

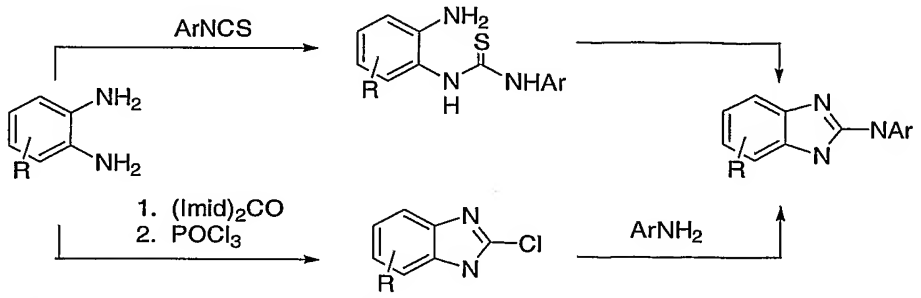
Various compounds and methods of their synthesis are disclosed in international patent application Publication Nos. WO02/18327 (benzamide and pyridylamide based compounds); WO0222598, and WO02/18383 (ABIQ based compounds); and WO 02/81443 (pthalamide base compounds), that have been found within context of this invention to be useful for immune potentiation. The entire disclosure of these U.S. and international publications is incorporated herein by this reference. Other compounds or intermediates of interest in the present invention were purchased from commercially available sources using the following method: the chemical structure of interest was drawn into the ACD-SC database (from MDL Information Systems). A search of the following companies/institutions, among others, retrieved the identified compound's supplier and purchasing information: ASDI, ASINEX, BIONET, CHEMBRIDGE, CHEMDIV, CHEMEX, CHEMSTAR, COMGENEX, CSC, INTERBIOSCREEN, LABOTEST, MAYBRIDGE, MICROSOURCE/GENESIS, OLIVIA, ORION, PEAKDALE, RYAN SCIENTIFIC, SPECS, TIMTEC, U OF FLORIDA, and ZELINSKY.

BENZAZOLE COMPOUNDS

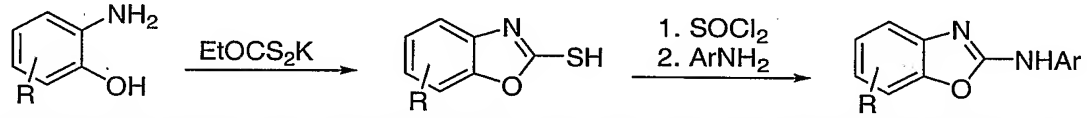
Scheme 1

Compounds of the invention containing a benzimidazole core may be prepared using a number of methods familiar to one of skill in the art. In one method, suitably functionalized diamines may be coupled with various thioisocyanates to form the intermediate thioureas. Cyclization to form the benzimidazole moiety may be effected under known conditions such as with treatment carbodiimides or alkyl halides. Alternatively the diamines may be reacted

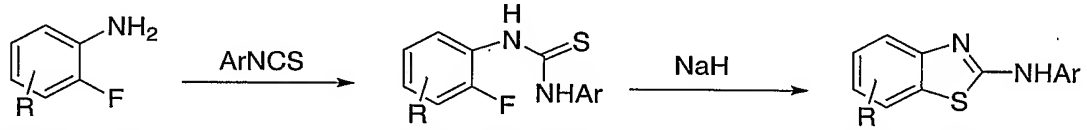
sequentially with carbonyl diimidazole and phosphoryl chloride followed by coupling with the appropriate amine.



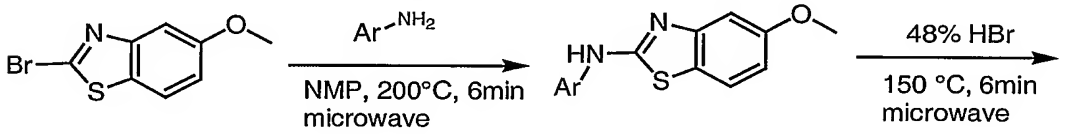
Compounds containing the oxazole structure may similarly be prepared according to the methods above or according to other known general procedures. Haviv et. al. (J. Med. Chem. 1988, 31, 1719) describes a procedure for assembling oxazole cores wherein a hydroxy aniline is treated with ethyl potassium xanthate. The resulting sulfuryl benzoxazole may then be chlorinated and coupled with an amine.

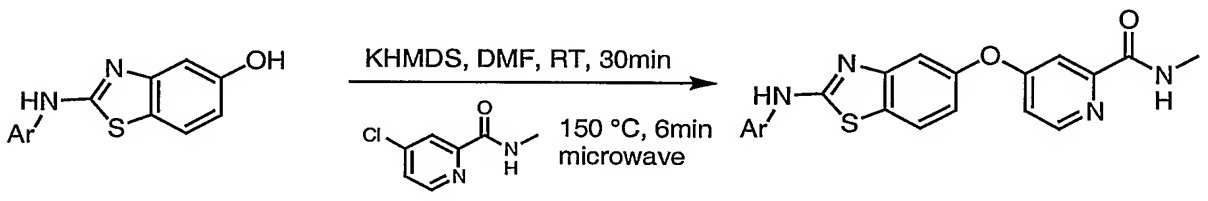


Compounds containing a benzothiazole core may also be prepared according to known methods. An ortho-halothioisocyanate may be reacted with an amine to form a thiourea. Reduction with NaH then allows formation of the thiazole ring.



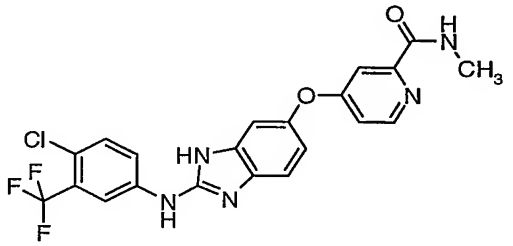
Benzothiazoles may generally be substituted in accordance with the present invention, such as through the following synthetic pathway:





Synthesis of 4-[(2-{[4-chloro-3-(trifluoromethyl)phenyl]amino}-1H-benzimidazol-6-yl)oxyl-N-methylpyridine-2-carboxamide

The compound 4-[(2-{[4-chloro-3-(trifluoromethyl)phenyl]amino}-1H-benzimidazol-6-yl)oxy]-N-methylpyridine-2-carboxamide (159322) was synthesized as follows:



Step 1. Synthesis of 4-[(4-amino-3-nitrophenyl)oxy]-*N*-methylpyridine-2-carboxamide: A mixture containing 4-amino-3-nitrophenol (1eq) and potassium bis(trimethylsilyl)amide (2eq) was stirred in dimethylformamide for 2 hours at room temperature. To this mixture was added (4-chloro(2-pyridyl))-*N*-methylcarboxamide (1eq) and potassium carbonate (1.2eq) and stirred at 90°C for 3 days. The reaction mixture was then concentrated and partitioned between ethyl acetate and water. The organic layer was separated and washed with brine, dried, filtered, and concentrated in vacuum to give brown solid. Purification on silica gel (2% triethyl amine / 50% ethyl acetate in hexane) gave 4-[(4-amino-3-nitrophenyl)oxy]-*N*-methylpyridine-2-carboxamide as an orange solid. The product gave satisfactory NMR. HPLC, 3.39min; MS: $MH^+ = 289$.

Step 2. Synthesis of 4-[(3,4-diaminophenyl)oxy]-*N*-methylpyridine-2-carboxamide: The mixture containing [4-(3-amino-4-nitrophenoxy)(2-pyridyl)]-*N*- in methanol with catalytic amount of 10%Pd/C was hydrogenated until disappearance of the yellow color to yield the product amine. HPLC, 2.5mins; MS: $MH^+ = 259$.

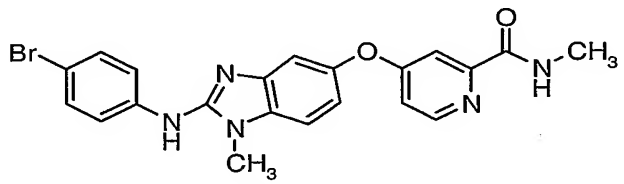
Step 3. Synthesis of 4-[(2-{[4-chloro-3-(trifluoromethyl)phenyl]amino}-1H-benzimidazol-6-yl)oxy]-N-methylpyridine-2-carboxamide:

The mixture containing 4-[(3,4-diaminophenyl)oxy]-*N*-methylpyridine-2-carboxamide (1eq) and 4-chloro-3-(trifluoromethyl)benzeneisothiocyanate (1eq) in tetrahydrofuran was stirred at room temperature for 16 hours to give the corresponding thiourea. To the resulting mixture was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (2eq) and the mixture was stirred for another 10 hours. The mixture was concentrated and partitioned between ethyl acetate and water. The organic layer was washed with brine and dried. Purification on HPLC gave 4-[(2-

{[4-chloro-3-(trifluoromethyl)phenyl]amino}-1H-benzimidazol-6-yl)oxy]-N-methylpyridine-2-carboxamide. MS: MH^+ = 462

Synthesis of 4-({2-[(4-bromophenyl)amino]-1-methyl-1H-benzimidazol-5-yl}oxy)-N-methylpyridine-2-carboxamide

The compound 4-({2-[(4-bromophenyl)amino]-1-methyl-1H-benzimidazol-5-yl}oxy)-N-methylpyridine-2-carboxamide (161651) was synthesized as follows:



Step 1. Synthesis of 4-{[3-amino-4-(methylamino)phenyl]oxy}-*N*-methylpyridine-2-carboxamide: A solution of 4-[(4-amino-3-nitrophenyl)oxy]-*N*-methylpyridine-2-carboxamide (1eq) in methylene chloride was treated with trifluoroacetic anhydride (1eq) and stirred for 10 minutes at 0 °C. The mixture was quenched with satd. $NaHCO_3$ solution. The organic layer was separated and washed with water, brine, dried and evaporated. MS: MH^+=385.2

To a solution of the trifluroacetamide (1eq) in a mixture of toluene, acetonitrile and sodium hydroxide solution (50%) was added benzyltrimethylammonium chloride (1eq) and dimethyl sulfate (1.2eq). The biphasic mixture was stirred overnight at room temperature and evaporated. The mixture was taken up in ethyl acetate, washed with water, brine, dried and evaporated. The crude product was purified by column chromatography eluting with 1:1 hexanes and ethylacetate followed by 2% triethylamine in 1:1 hexanes and ethyl acetate followed by 2% triethylamine in 1:1 hexanes and ethyl acetate to afford *N*-methyl-4-{[4-(methylamino)-3-nitrophenyl]oxy}pyridine-2-carboxamide as a reddish orange solid. MS: MH^+ = 303.1.

The solution of nitromethylaniline in methanol was treated with 5% palladium on carbon and stirred under hydrogen atmosphere for 15 min. (until the disappearance of yellow coloration) at room temperature. The mixture was filtered and the filtrate was concentrated to provide 0.36 g of the diamine 4-{[3-amino-4-(methylamino)phenyl]oxy}-*N*-methylpyridine-2-carboxamide. MS: MH^+ = 273.3.

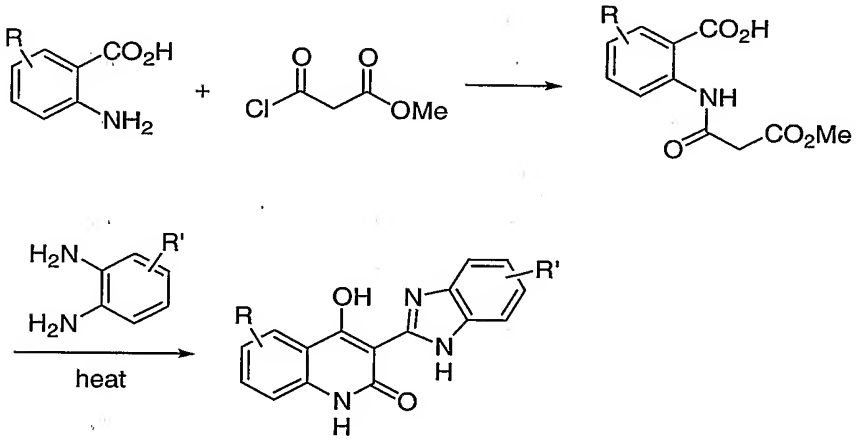
Step 2. Synthesis of 4-({2-[(4-bromophenyl)amino]-1-methyl-1H-benzimidazol-5-yl}oxy)-N-methylpyridine-2-carboxamide: A solution of the diamine 4-{[3-amino-4-(methylamino)phenyl]oxy}-*N*-methylpyridine-2-carboxamide (1eq) in methanol was treated with 4-bromophenylisothiocyanate (1eq) and stirred at 60 °C - 65°C for 2 hours. The reaction mixture was cooled down to room temperature and methyl iodide (1eq) was added and stirred overnight at 60°C. The reaction was cooled to room temperature, evaporated, taken up in ethyl

acetate, and washed with water and brine, dried, and evaporated under reduced pressure. Column chromatography using a gradient solvent system of hexanes and ethyl acetate and either 1:1 methylene chloride and acetone or 5% methanol in methylene chloride yielded the product as a half white powder. MS: MH^+=452.3

AMINOBENZIMIDAZOLYLQUINOLINONES

Compounds of structure I may be synthesized from simple starting molecules as shown in Schemes 1-4 and exemplified in the Examples. As shown in Scheme 1, compounds of structure I may generally be prepared using aromatic compounds substituted with amines and carboxylic acid groups.

Scheme 2.

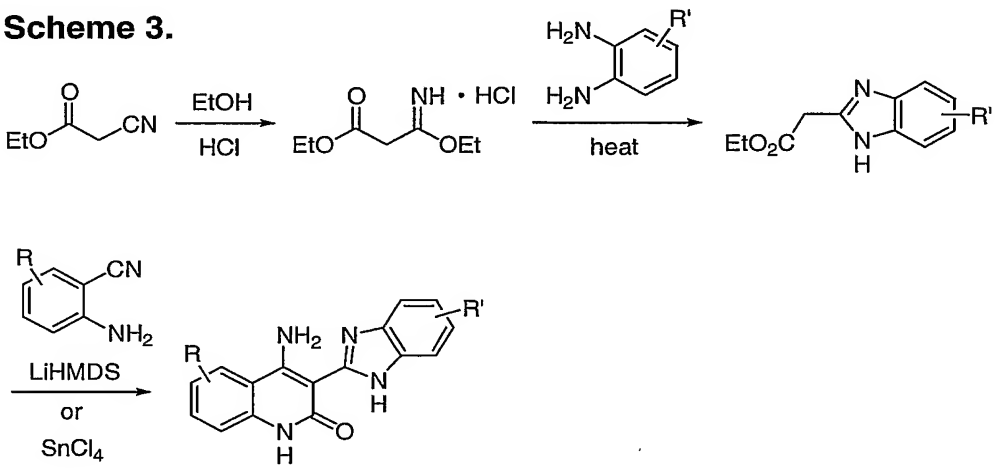


As shown in Scheme 2, a substituted aromatic compound such as a substituted or unsubstituted 2-aminobenzoic acid may be reacted with an acyl halide such as methyl 2-(chlorocarbonyl)acetate to produce an amide that will react with a substituted or unsubstituted 1,2-diaminobenzene. The resulting product is a 4-hydroxy-substituted compound of structure I. One skilled in the art will recognize that the procedure set forth in Scheme 1 may be modified to produce various compounds.

A method for preparing 4-amino substituted compounds of structure I is shown in Scheme 3. As shown in Scheme 3, aromatic compounds substituted with amine and nitrile groups may be used to synthesize 4-amino substituted compounds of structure I. A compound such as ethyl 2-cyanoacetate may be reacted with ethanol to produce ethyl 3-ethoxy-3-iminopropanoate hydrochloride. Subsequent reaction with a substituted or unsubstituted 1,2-phenylenediamine provides substituted or unsubstituted ethyl 2-benzimidazol-2-ylacetate. Reaction of a substituted or unsubstituted ethyl 2-benzimidazol-2-ylacetate with an aromatic

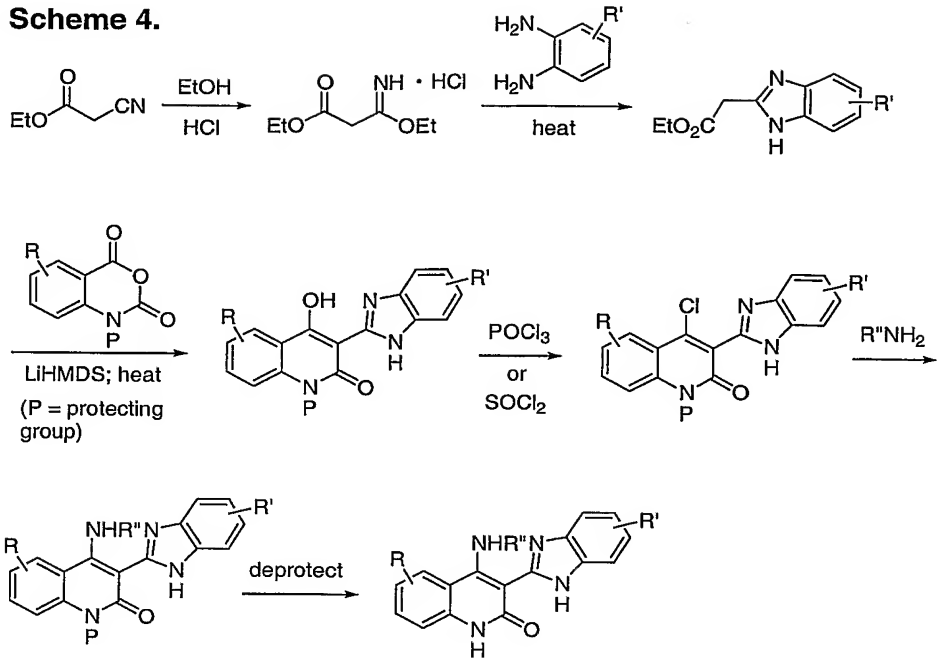
compound having an amine and nitrile group such as substituted or unsubstituted 2-aminobenzonitrile with a base such as lithium bis(trimethylsilyl)amide or a Lewis acid such as tin tetrachloride provides the substituted or unsubstituted 4-amino substituted compound of structure I.

Scheme 3.



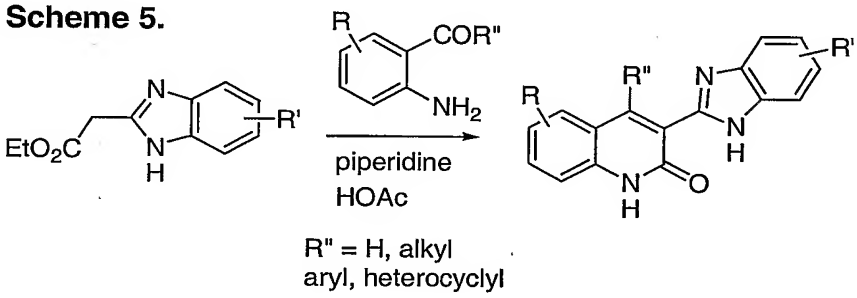
Scheme 4 illustrates a general synthetic route that allows for the synthesis of 4-dialkylamino and 4-alkylamino compounds of structure I. An inspection of Scheme 3 shows that 4-hydroxy substituted compounds of structure I may be converted into the 4-chloro derivative by reaction with phosphorous oxychloride or thionyl chloride. The 4-chloro derivative may then be reacted with an alkylamine or dialkylamine to produce the corresponding 4-alkylamino or 4-dialkylamino derivative. Deprotection affords the final 4-alkylamino or 4-dialkylamino compounds of structure I. Other groups that may be reacted with the 4-chloro derivative in this manner include, but are not limited to, ROH, RSH, and CuCN.

Scheme 4.



As shown in Scheme 5, the synthesis of compounds of structure I having a H, alkyl group, aryl group, or heterocyclyl group in the 4-position may be accomplished using a substituted or unsubstituted 2-benzimidazol-2-ylacetate prepared as shown in Schemes 3 and 4.

Scheme 5.

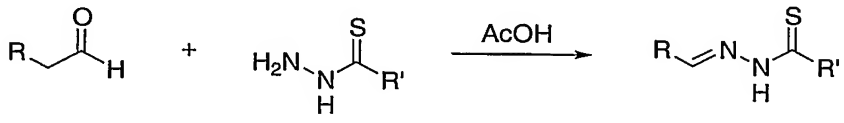


R" = H, alkyl
aryl, heterocyclyl

THIOSEMCARBAZONES

General procedure for the preparation of thiosemicarbazones

Scheme 6



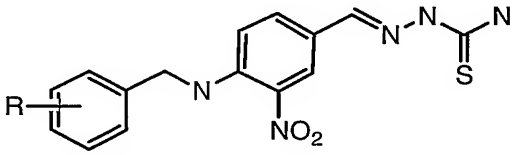
A solution of aldehyde (1.0 equiv.) and thiosemicarbazide (1.05 equiv.) in acetic acid was stirred overnight. Excess of acetic acid was removed to give a residue, that was washed with ethanol, or purified by preparative-HPLC to give the thiosemicarbazone.

Scheme 7

A solution of aldehyde (1.0 equiv.), thiosemicarbazide (1.05 equiv.) and acetic acid (0.1 equiv.) in methanol was stirred overnight. Methanol was removed to give a residue, that was worked up as in Scheme 6.

Scheme 8

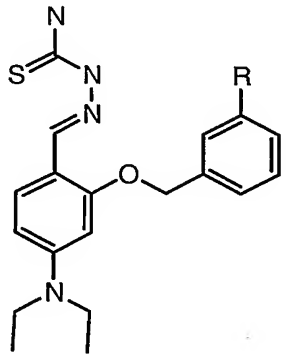
To a solution of {[(1E)-1-aza-2-(4-fluoro-3-nitrophenyl)vinyl]amino}-aminomethane-1-thione in ethanol was added an arylamine (2.1 equiv.). The solution was stirred at room temperature until the starting fluoride disappeared. The solution was purified to the product.



Scheme 9

A mixture of 4-(diethylamino)-2-hydroxybenzaldehyde (1 equiv.), benzylic bromide (1.2 equiv.) and powder potassium carbonate in ethanol was stirred at room temperature for 2 days. Ethanol was removed, and the residue was dissolved in ethyl acetate and water. The organic layer was washed with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4., and concentrated. The residue was purified on silica gel eluting with ethyl acetate/hexane to give 4-(diethylamino)-2-benzoxylic-benzaldehyde.

The aldehydes were converted to thiosemicarbazones according to Scheme 7.



Scheme 10

A solution of 3,4-difluorobenzenecarbonitrile (1 equiv.), amine (1.5 equiv.) and DIEA (2 equiv.) in NMP was heated in a Smith Microwave (Personal Chemistry) for 30 minutes. The reaction mixture was purified on silica gel to give 4-substituted 3-fluorobenzenecarbonitrile.

To a solution of nitrile in toluene at –78 °C was added DIBAL-H (1 M in toluene, 1.5 equiv.). The reaction mixture was warmed to rt, and stirred for 16 h, and quenched with methanol/ethyl acetate/brine (1:1:4). After being stirred at rt for 30 min, the solution was extracted with ethyl acetate (3x). The combined organic layers were washed with aqueous $NaHCO_3$, brine and concentrated. The aldehyde was purified on silica gel or directly converted to thiosemicarbazones (Scheme 7).

Scheme 11

A solution of 2,4,5-trifluorobenzenecarbonitrile (1 equiv.) and 4-arylpiperazine (1.2 equiv.) and DIEA (1.2 equiv.) in THF was heated at 80 °C for 2 hours. The mixture was purified on silica gel to give 4-substituted 2,5-difluorobenzenecarbonitrile.

Scheme 12

To an alcohol (1.0 equiv) was added potassium *t*-butoxide in THF (1 M, 1.1 equiv). After 5 minutes, the solution was added to a solution of 4-N-substituted-2,5-difluorobenzenecarbonitrile (1 equiv.) in THF. The reaction mixture was stirred at rt overnight and quenched with aqueous ammonium chloride. The aqueous layer was extracted with ethyl acetate (3x). The combined organic layers were washed with brine, and concentrated to give a residue, that was purified to give 4-N-substituted-2-O-substituted-5-fluorobenzenecarbonitrile.

4-N-substituted-2-O-substituted-5-fluorobenzenecarbonitrile was reduced with DIBAL-H to give a 4-N-substituted-2-O-substituted-5-fluorobenzaldehyde according to procedure in Scheme 10.

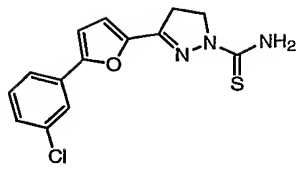
The aldehyde was converted to the corresponding thiosemicarbazone using Scheme 7.

Scheme 13

A solution of 4-N-substituted-2,5-difluorobenzenecarbonitrile (1 equiv.), amine (1.5 equiv.) and DIEA (2 equiv.) in NMP was heated in a Smith Microwave (Personal Chemistry) for 30 minutes. The reaction mixture was purified on silica gel to give 4-N-substituted-2-N-substituted-5-fluorobenzenecarbonitrile.

4-N-substituted-2-N-substituted-5-fluorobenzenecarbonitrile was reduced with DIBAL-H according to procedure described in Scheme 10 to give 4-N-substituted-2-N-substituted-5-fluorobenzaldehyde.

Preparation of amino{3-[5-(3-chlorophenyl)(2-furyl)](2-pyrazolinyl)}methane-1-thione



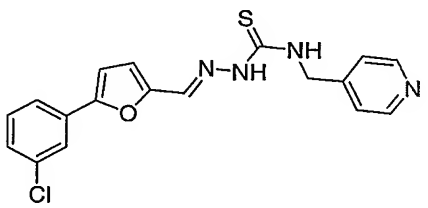
To a solution of 5-(3-chlorophenyl)furan-2-carbaldehyde (1.0 equiv.) in THF at 0 °C was added MeMgBr in ether (3.0 equiv.) and stirred for 45 min. The reaction was quenched with water, diluted with ether and filtered through Celite. The organic layer was separated and washed with brine, dried over $MgSO_4$, and concentrated to give the 1-[5-(3-chlorophenyl)-2-furyl]ethan-1-ol.

To a solution of secondary alcohol(1.0 equiv.) in CH_2Cl_2 was added MnO_2 (10 equiv.). The reaction was stirred overnight, filtered through Celite, and concentrated to give 1-[5-(3-chlorophenyl)-2-furyl]ethan-1-one.

To a mixture of ketone (1.0 equiv.), paraformaldehyde (2.0 equiv.), and dimethylamine hydrochloride (2.0equiv) and molecular sieves in ethanol was added concentrated hydrochloric acid (cat.). The reaction was refluxed overnight under nitrogen and the concentrated. A few drops of HCl was added, and the mixture was worked up with DCM and water. The organic layer was discarded. The aqueous layer was adjusted to basic and extracted with DCM (3x). The organic layer was washed with brine, dried over $MgSO_4$, and concentrated to yield 3-(dimethylamino)-1-[5-(3-chlorophenyl)(2-furyl)]propan-1-one.

Thiosemicarbazide (1.0 equiv.) was dissolved in MeOH upon heating under nitrogen. Aqueous sodium hydroxide (6 M, 9.0 equiv.) was added to the reaction. A methanol solution of 3-(dimethylamino)-1-[5-(3-chlorophenyl)(2-furyl)]propan-1-one (1.0 equiv) was then added dropwise to the reaction mixture. The solvent was removed and the residue was dissolved in DCM and washed with water, brine, dried over $MgSO_4$, and concentrated. The final compound was purified by preparative-HPLC to give amino{3-[5-(3-chlorophenyl)(2-furyl)](2-pyrazolinyl)}methane-1-thione; LC/MS m/z 306.2 (MH+); Rt =3.06 minutes .

Scheme 14



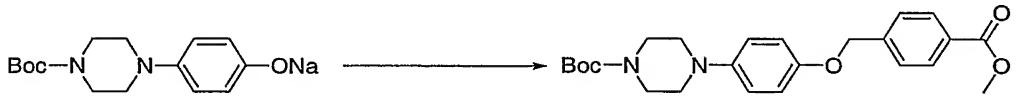
To a solution of 4-pyridylmethylamine (1.0 equiv.) and triethylamine (2.0 equiv.) in $CHCl_3$ was added CS_2 (1.0 equiv.)) and stirred overnight. The reaction was cooled to 0 °C and ethyl chloroformate (1.0 equiv.) was added dropwise. The reaction was stirred for 15 min at 0 °C and then stirred at room temperature for 2 hrs followed by addition of (tert-butyl)oxycarbohydrazide (1.2 equiv.). After stirring for an addition hour the mixture was washed with aqueous citric acid (5%), saturated $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated. The desired Boc protected thiosemicarbazide was purified using column chromatography.

To a solution of Boc protected thiosemicarbazide (1.0 equiv.) dissolved in DCM was added HCl in dioxane (2M, 8.3 equiv.) and stirred for 15 min. MeOH is then added to dissolve the precipitate, followed by addition of the furfural, and small amount of acetic acid (0.5 mL). The mixture is stirred overnight and the solvents are removed to give a residue purified by preparative-HPLC to give the thiosemicarbazone.

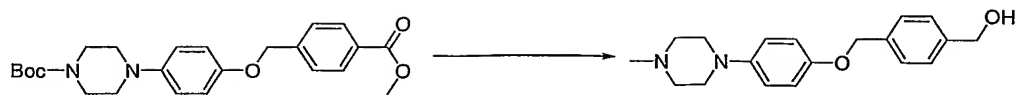
Synthesis of 4-[4-(4-methylpiperazin-1-yl)phenoxymethyl]benzaldehyde

To a solution of 4-piperazin-1-yl phenol (1 equivalent) in $CHCl_3$, cooled to 0 °C, was added di-*t*-butyl dicarbonate (1 equivalent) in $CHCl_3$ drop-wise. The solution was stirred at 0 °C for 1 hour before removing from the cold bath and stirring at ambient temperatures for 18 hours. The organic solution was washed aqueous $NaHCO_3$ and brine dried over $MgSO_4$ and concentrated the crude material was used without purification.

A solution of the resulting 4-(1-BOC-piperazin-4-yl)phenol (1 equivalent) in dry CH_3CN was slowly added drop-wise to a slurry of NaH (1 equivalent) in dry CH_3CN at room temperature under N_2. The slurry was stirred at room temperature for 2 hours before the solids were filtered and washed with Et_2O.



Sodium 4-(1-BOC-piperazin-4-yl)phenoxide (1 equivalent) and methyl 4-bromomethylbenzoate (1 equivalent) were combined in dry acetone and heated to reflux at 60 °C for 18 hours. The slurry was filtered and the filtrate was then concentrated to provide the crude methyl 4-[4-(1-BOC-piperazin-4-yl)phenoxymethyl]benzoate, that was used without purification.



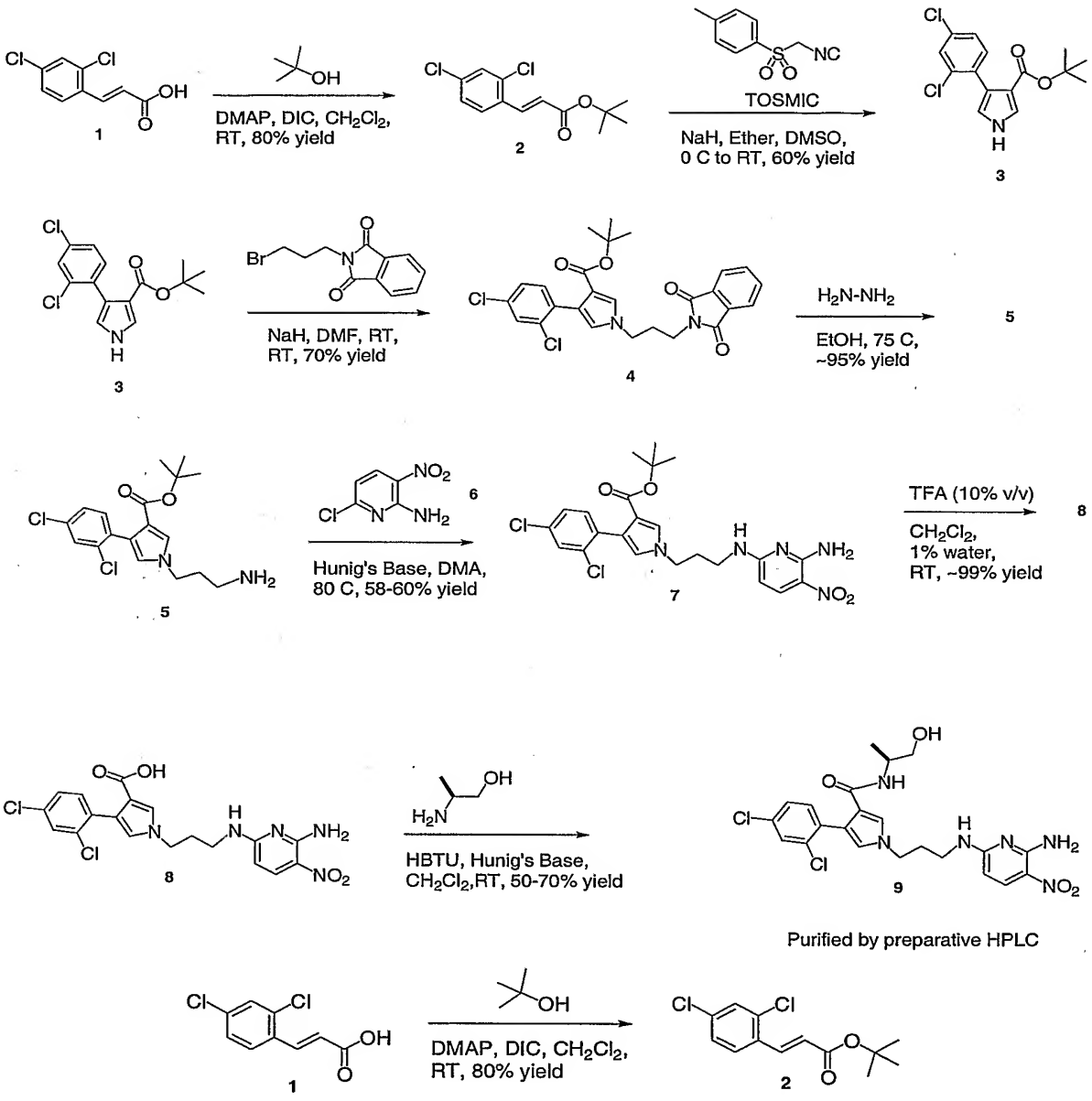
To a slurry of $LiAlH_4$ (4 equivalents) in dry THF, cooled to 0 °C under N_2, was slowly added drop-wise a solution of methyl 4-[4-(1-BOC-piperazin-4-yl)phenoxymethyl]benzoate (1 equivalent) in dry THF. Once the addition was complete, the slurry was heated to reflux at 80 °C for 1 hour. The slurry was subsequently cooled to 0 °C and treated with water, 10% aq. NaOH and with water again. The resulting solids were filtered, and the filtrate was diluted with chloroform, washed with brine, dried over $MgSO_4$ and concentrated, providing the crude 4-[4-(4-methylpiperazin-1-yl)phenoxymethyl]benzyl alcohol that was used without purification.

To a solution of DMSO (2.6 equivalents) in dry DCM , cooled to –78 °C under N_2 was added oxalyl chloride (1.1 equivalents) in DCM drop-wise. The solution was stirred at –78 °C for 5 minutes before a solution of 4-[4-(4-methylpiperazin-1-yl)phenoxymethyl]benzyl alcohol (1 equivalent) in DCM was added drop-wise, and allowed to stir at –78 °C for another 30 minutes. Triethylamine (2.5 equivalents) was slowly dripped in before allowing the solution to reach ambient temperatures. The solution was washed with aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated to provide the crude 4-[4-(4-methylpiperazin-1-yl)phenoxymethyl]benzaldehyde that was converted to thiosemicarbazones according to Scheme 7.

PYRROLES

Scheme 15

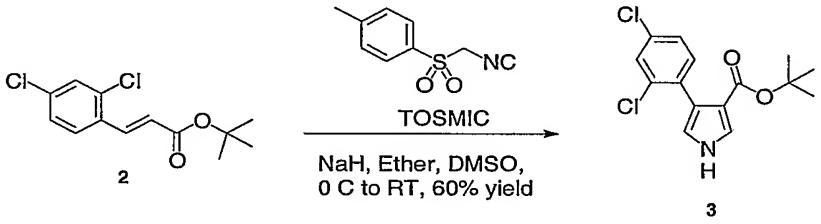
Synthesis of Pyrrole



Preparation of *tert*-butyl (2E)-3-(2,4-dichlorophenyl)prop-2-enoate (2).

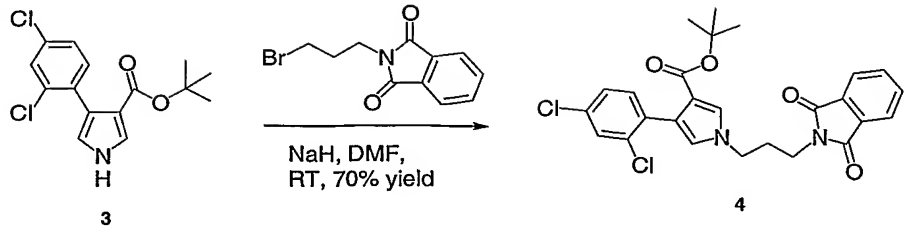
Neat DIC (1.4 eq) was added to a well stirred solution of cinnamate (1 eq), t-butyl alcohol (4 eq), DMAP (1.4 eq) and CH_2Cl_2 under argon at rt. (Note - The cinnamate must be completely in solution that may require gentle warming. Allow the solution to cool to room temperature before adding the DIC. To avoid an exotherm on larger scales, it may be prudent to

dilute the DIC with CH_2Cl_2 before the addition and have an ice bath ready.) After stirring for 8 hours, the reaction develops a white precipitate. The reaction may be monitored by TLC eluting with 25% EtOAc/Hexane (R_f of product was 0.9). The entire reaction was loaded into a separatory funnel (washing with CH_2Cl_2). The organic mixture was washed with citrate, sat. aq. $NaHCO_3$, water, and brine. The organic layer was dried (Na_2SO_4), filtered, and concentrated to dryness to give the crude product as an oil. The crude oil was mixed with hexane and stirred for 30 min. The precipitate that forms was filtered over celite and the filtrate was evaporated. The hexane mixture was loaded onto a filter plug of silica and eluted with EtOAc/hexane (97:2 v/v). The first eluted UV active fractions are collected and evaporated to give >99% pure **2** (75-80% yields).



Preparation of *tert*-butyl 4-(2,4-dichlorophenyl)pyrrole-3-carboxylate (3).

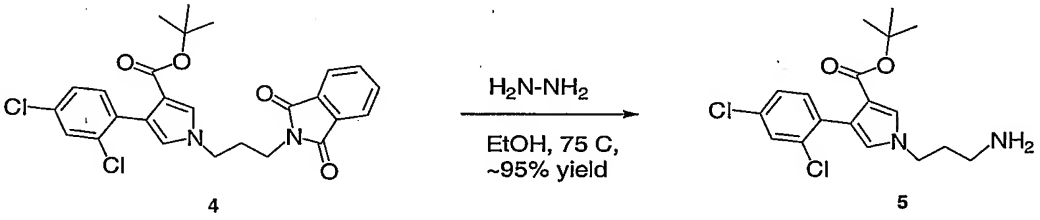
Dry ether was added to NaH (1.5 eq as the oil dispersion) under argon. After decanting off the ether via syringe, the NaH was suspended again with fresh ether under argon. A solution of TOSMIC (1.1 eq) and **2** (1 eq) dissolved in a mixture of ether and DMSO was added dropwise to the stirred suspension of NaH at 0 °C over 20-30 min. The addition was mildly exothermic and evolved gas. After the addition, the reaction was allowed to warm to ambient rt. The progress of the reaction was followed by TLC (25% EtOAc/Hexane, the UV active product was at $R_f = 0.4$) and LCMS until done (~2-3 h). Upon completion, the reaction was carefully quenched with sat. aq. NH_4Cl (added slowly to avoid strong gas evolution and exotherm) and diluted with ether. The layers were separated and the organic phase was washed with sat. aq. $NaHCO_3$, water, and brine. The crude dark solid can be purified by recrystallization. Best results were achieved either through recrystallization directly from a mixture of hot EtOAc/hexane (1:3 v/v) or by dissolving the crude product in minimal hot EtOAc followed by addition of hexane (~2 volumes of hexane based on the volume of EtOAc). The hot solutions were allowed to cool to room temperature and age over night. The crystals were first filtered and then washed with hexane giving 99% pure product in 60-70 % yield.



3 4

Preparation of *tert*-butyl 4-(2,4-dichlorophenyl)-1-[3-(1,3-dioxobenzo[c]azolin-2-yl)propyl]pyrrole-3-carboxylate (4).

Solid NaH (1.5 eq as the oil dispersion) was added in small portions to a solution of pyrrole **3** (1 eq) and 3-bromopropyl phthalimide (1.2 eq) dissolved in DMF stirred at room temperature and flushed with argon. NOTE - Some gas evolves, but the temperature does not seem to rise above 40-50 °C. The reaction was stirred for 1.5 h at room temperature under argon. The reaction was followed by TLC (CH_2Cl_2/acetonitrile (95:5 v/v), the UV active product was at R_f= 0.5) and LCMS. Upon completion, the reaction was quenched with sat. aq. NH_4Cl (add slowly to avoid strong gas evolution and exotherm). Sat. aq. $NaHCO_3$ was then added to avoid an emulsion, and the basic organic mixture was extracted with ether. The combined ether layers were washed with sat. aq. $NaHCO_3$, water, brine, dried Na_2SO_4, filtered, and concentrated to dryness to give the crude product. The crude product was purified by eluting through silica with EtOAc/Hexane (1:4 v/v). The purified product contained some residual 3-bromopropyl phthalimide, that did not interfere with subsequent synthetic steps. The material was taken on and used without further purification. Assume a quantitative yield.

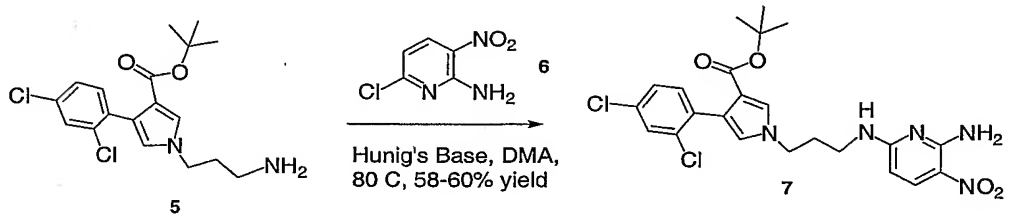


4 5

Preparation of *tert*-butyl 1-(3-aminopropyl)-4-(2,4-dichlorophenyl)pyrrole-3-carboxylate (5).

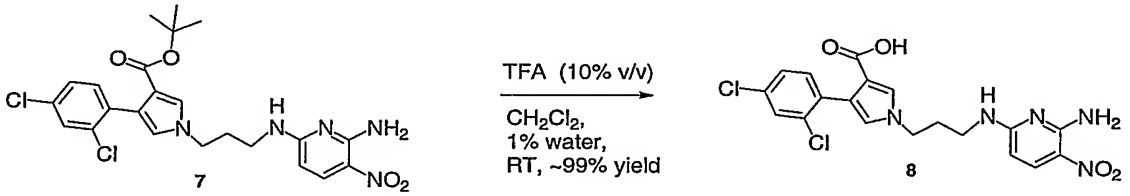
The Pthalimido Pyrrole **4** (1 eq) was dissolved in ethanol and hydrazine (3 eq) at room temperature under nitrogen. Upon heating to reflux, the reaction generated a white precipitate. Stir at reflux until complete (~2 h) by TLC (CH_2Cl_2/acetonitrile (95:5 v/v), the UV active product was at R_f= 0.2) and LCMS. Upon reaching completion, the reaction was allowed to cool to room temperature and the precipitate was vacuum-filtered off using a medium to fine cintered-glass filter. The filtrate was concentrated under reduced pressure to a gummy solid.

The crude material was taken up in ethanol/EtOAc (1:1 v/v), stirred and the precipitate was filtered off in the same fashion as before. The filtrate was concentrated under reduced pressure and than dried *in vacuo* for 10-15 min. This process of adding ethanol/EtOAc, filtering and concentrating was done one more time or as needed to remove the majority of the white precipitate and residual hydrazine. The product was then dried *in vacuo* overnight. The material was used without further purification. Once dried, the reaction yielded the product as a glass (~87% yield over 2 steps).



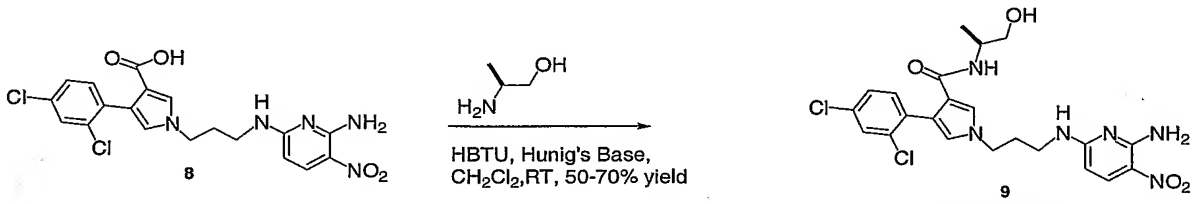
Preparation of *tert*-butyl 1-{3-[(6-amino-5-nitro(2-pyridyl))amino]propyl}-4-(2,4-dichlorophenyl)pyrrole-3-carboxylate (7).

To the premixed dry reagents, pyrrole **5** (1 eq) and powdered 6-chloro-3-nitro-2-pyridylamine (**6**) (1.1 eq), was added the DMA followed by Hünig's base (2 eq) sequentially with stirring at rt. The reaction was then heated to 80 °C overnight. The reaction was followed by TLC (EtOAc/hexane (1:1 v/v), the UV active yellow product was at R_f= 0.25), HPLC and LCMS. Upon completion as judged by HPLC, the reaction was allowed to cool to 70 °C. Ethylene diamine (anhydrous) was then added to the reaction to destroy any remaining unreacted chloropyridine **6**. After 15 min stirring at 70 °C, the reaction was cooled and quenched with the addition of sat. aq. $NaHCO_3$. The aqueous mixture was extracted with EtOAc, and the combined organic layers were washed with sat. aq. $NaHCO_3$, water, brine, dried, filtered, and concentrated to dryness to give the crude product as a brown-yellow solid. The crude product was purified by flash chromatography eluted with EtOAc/hexane (4:6 v/v). The purified SnAr adduct **7** was isolated in 58% yield as a yellow solid.



Preparation of 1-{3-[(6-amino-5-nitro(2-pyridyl))amino]propyl}-4-(2,4-dichlorophenyl)pyrrole-3-carboxylic acid (8).

In a vial, TFA (catalytic amount) was added to a stirred mixture of *tert*-butyl ester pyrrole 7 (1 eq), water (.1%), and CH_2Cl_2 at rt. The vial stirred at room temperature until done (~12 h. The reaction was then concentrated under reduced pressure at room temperature and dried *in vacuo*. The crude residue was dissolved again in CH_2Cl_2 and concentrated under reduced pressure at rt. The material was used in the final coupling step without further purification as the TFA salt.



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Preparation of *N*-((1S)-2-hydroxy-isopropyl)(1-{3-[(6-amino-5-nitro(2-pyridyl))amino]propyl}-4-(2,4-dichlorophenyl)pyrrol-3-yl)carboxamide (9,).

(2S)-(+)-2-Aminopropan-1-ol (1.5 eq) was added to a stirred mixture of acid (**8**) (1 eq), HBTU (1.5 eq), Hünig's base (2 eq) and DMF (premixed sequentially in this order in a vial) at room temperature under argon. The reaction was stirred for 3-4 h until complete as shown by LCMS and HPLC. The reaction mixture was subsequently diluted with EtOAc, washed with $NaHCO_3$, and concentrated to afford a powder in a 70% yield.

Nomenclature for the Example compounds was provided using ACD Name version 5.07 software (November 14, 2001) available from Advanced Chemistry Development, Inc. Some of the compounds and starting materials were named using standard IUPAC nomenclature.

The compounds of Table 34 were synthesized following the synthetic methodology described above in the Examples and Schemes, and screened following methods 1 and 2 below. The precursors are readily recognizable by one skilled in the art and are commercially available from Aldrich (Milwaukee, WI) or Acros Organics (Pittsburgh, PA), among others.

Screening methods for SMIP/SMIS compounds

Method 1

Candidate small molecule immuno-potentiators can be identified *in vitro*. Compounds are screened *in vitro* for their ability to activate immune cells. One marker of such activation is the induction of cytokine production, for example TNF-α production. Apoptosis inducing small

molecules may be identified having this activity. These small molecule immuno-potentiators have potential utility as adjuvants and immuno-therapeutics.

In an assay procedure (High Throughput Screening (HTS)) for small molecule immune potentiators (SMIPs), human peripheral blood mononuclear cells (PBMC), 500,000 per mL in RPMI 1640 medium with 10% FCS, were distributed in 96 well plates (100,000 per well) already containing 5μM of compound in DMSO. The PBMCs were incubated for 18 h at 37°C in 5% CO_2. Their ability to produce cytokines in response to the small molecule compounds is determined using a modified sandwich ELISA.

Briefly supernatants from the PBMC cultures were assayed for secreted TNF using a primary plate bound antibody for capture followed by a secondary biotinylated anti-TNF antibody forming a sandwich. The biotinylated second antibody was then detected using streptavidin-Europium and the amount of bound europium was determined by time resolved fluorescence. SMIP compounds were confirmed by their TNF inducing activity that was measured in the assay as increased Europim counts over cells incubated in RPMI medium alone. "Hits" were selected based on their TNF-inducing activity relative to an optimal dose of lipopolysaccaride LPS (1 μg/ml), a strong TNF inducer. The robustness of the assay and low backgrounds allowed for the routine selection of hits with ~10% of LPS activity that was normally between 5-10X background (cells alone). Selected hits are then subjected to confirmation for their ability to induce cytokines from multiple donors at decreasing concentrations. Those compounds with consistent activity at or below 5μM are considered confirmed for the purposes of this assay. The assay is readily modified for screening for compounds effective at higher or lower concentrations.

Method 2

Each of the compounds in the above Table 34 elicited TNF-α production in human peripheral blood mononuclear cells. Many of the compounds showed activity at less than 20 μM with respect to production of TNF-α. Many of these compounds showed activity at less than 5 μM with respect to production of TNF-α. Many of these compounds showed activity in the production of TNF-α at less than 1.5 μM.

For this reason, each of the R groups of any of the compounds listed in Table 34 are preferred. Additionally, because of the excellent activity of each of the compounds, each of these compounds is individually preferred and is preferred as a member of a group that includes any or all of the other compounds and each compound is preferred in methods of modulating

immunopotentiation and in methods of treating biological conditions associated therewith, for example to be used as a vaccine adjuvant. Each of the compounds is also preferred for use in preparation of medicaments for vaccines, immunopotentiation, reducing tumor growth and in treating biological conditions mediated therefrom.

In additon to the procedure described above, methods of measuring other cytokines (e.g. IL1-beta, IL-12, IL-6, IFN-gamma, IL-10 etc.) are well known in the art and can be used to find active SMIP compounds of the present invention.

Compounds may be useful that cause production of TNF-α at higher concentrations, such as 100μM, 200 μM or 300μM in the assays described herein. For example Loxoribine causes useful production of TNF-α at 300μM (see Pope et al Immunostimulatory Compound 7-Allyl-8-Oxoguanosine (Loxoribine) Induces a Distinct Subset of Murine Cytokines Cellular Immunology 162: 333-339 (1995)).

The subject invention also includes isotopically-labeled antiviral compounds, that are structurally identical to those disclosed above, but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into antiviral compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, sulfur, fluorine and chlorine, such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F and ^{36}Cl, respectively. Antiviral compounds of the present invention, derivatives thereof, and pharmaceutically acceptable salts of said compounds and of said derivatives that contain the aforementioned isotopes and/or other isotopes of other atoms are within the scope of this invention. Certain isotopically-labeled antiviral compounds of the present invention, for example those into which radioactive isotopes such as ^{3}H and ^{14}C are incorporated, are useful in drug and/or substrate tissue distribution assays. Tritiated, *i.e.*, ^{3}H, and carbon-14, *i.e.*, ^{14}C, isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium, *i.e.*, ^{2}H, may afford certain therapeutic advantages resulting from greater metabolic stability, for example increased *in vivo* half-life or reduced dosage requirements and, hence, may be preferred in some circumstances. Isotopically labeled antiviral compounds of this invention and derivatives thereof can generally be prepared by carrying out known or referenced procedures and by substituting a readily available isotopically labeled reagent for a non-isotopically labeled reagent.

In accordance with the present invention, methods are provided for the administration of an effective amount of a SMIP compound to act as an adjuvant. Also provided are immunogenic compositions comprising a SMIP compound, an antigen, and optionally other adjuvants.

As adjuvants, the SMIP compounds are combined with antigens and delivery systems to form a final immunogenic composition or vaccine product.

As immunotherapeutics, the SMIP compounds are used alone or in combination with other therapies for treatment of SARS.

Those of ordinary skill in the art will recognize that physiologically active antiviral compounds, SMIPs or SMISs that have accessible hydroxy groups are frequently administered in the form of pharmaceutically acceptable esters. The antiviral compounds of this invention can be effectively administered as an ester, formed on the hydroxy groups, just as one skilled in pharmaceutical chemistry would expect. It is possible, as has long been known in pharmaceutical chemistry, to adjust the rate or duration of action of the antiviral compound by appropriate choices of ester groups.

Other compounds that can be used in combination with the therapeutic agents described herein include, pentoxifylline (PTX), methylprednisolone, trimetrexate (Neutrexin), Zadaxin (thymosin alpha 1), optionally substituted 5-aminomethinimino-3-methyl-4-isoxazolecarboxylic acid phenylamides, cyclosporine A (CsA), 6-oxo-1,4,5-thiadiazin[2,3-*b*]quinazoline, 3-amino-2(1*H*)-thioxo-4(3*H*)-quinazolinone, gangciclovir, glycyrrhizin, tetracyclines, aminoglycosides, quinolones, bicyclam (1,4-Bis(1,4,8,11-tetraazacyclotetradec-1-ylmethyl)benzene octahydrochloride dihydrate), rapamycin, wortmannin, enalapril, roquinimex/linomide, inactivin, DNCB, AG7088, 9-aminocamptothecin (CPT-11) , loxorobine, bropirimine, Ononase ® (ranpirnase), statins, such as: lovastatin--Mevacor®, pravastatin--Pravachol®, simvastatin--Zocor®, fluvastatin--Lescol®, atorvastatin—Lipitor® and rosuvastatin--Crestor®.

As used herein, the term "effective amount" means an amount of antiviral compound of the compositions, kits and methods of the present invention that is capable of treating the symptoms of the described conditions. The specific dose of a compound administered according to this invention will, of course, be determined by the particular circumstances surrounding the case including, for example, the compound administered, the route of administration, the state of being of the patient, and the severity of the condition being treated.

The dose of an antiviral compound of this invention to be administered to a subject is rather widely variable and subject to the judgment of the attending physician. It should be noted that it may be necessary to adjust the dose of a compound when it is administered in the form of a salt, such as a laureate, the salt forming moiety of which has an appreciable molecular weight.

The following dosage amounts and other dosage amounts set forth elsewhere in this description are for an average human subject having a weight of about 65 kg to about 70 kg. The skilled practitioner will readily be able to determine the dosage amount required for a subject whose weight falls outside the 65 kg to 70 kg range, based upon the medical history of the

subject and the presence of diseases, *e.g.*, diabetes, in the subject. Calculation of the dosage amount for other forms of the free base form such as salts or hydrates is easily accomplished by performing a simple ratio relative to the molecular weights of the species involved.

In general, the pharmaceutical compositions will include at least one antiviral compound in combination with a pharmaceutically acceptable vehicle, such as saline, buffered saline, 5% dextrose in water, borate-buffered saline containing trace metals or the like. Formulations may further include one or more excipients, preservatives, solubilizers, buffering agents, lubricants, fillers, stabilizers, *etc.* Methods of formulation are well known in the art and are disclosed, for example, in "Remington's Pharmaceutical Sciences," Mack Pub. Co., New Jersey (1991) or "Remington: The Science and Practice of Pharmacy," 20th ed., Lippincott Williams & Wilkins, Baltimore, Maryland (2000), incorporated herein by reference.

Pharmaceutical compositions for use within the present invention can be in the form of sterile, non-pyrogenic liquid solutions or suspensions, coated capsules, suppositories, lyophilized powders, transdermal patches or other forms known in the art.

Many of the active ingredient antiviral compounds are known to be absorbed from the alimentary tract, and so it is usually preferred to administer a compound orally for reasons of convenience. However, the compounds may equally effectively be administered intravenously, subcutaneously, percutaneously, or as suppositories for absorption by the rectum or vagina, if desired in a given instance. All of the usual types of compositions may be used, including tablets, chewable tablets, capsules, solutions, parenteral solutions, troches, suppositories and suspensions. Compositions are formulated to contain a daily dose, or a convenient fraction of daily dose, in a dosage unit, that may be a single tablet or capsule or convenient volume of a liquid.

Capsules are prepared by mixing the compound or compounds with a suitable diluent and filling the proper amount of the mixture in capsules. The usual diluents include inert powdered substances such as starch of many different kinds, powdered cellulose, especially crystalline and microcrystalline cellulose, sugars such as fructose, mannitol and sucrose, grain flours and similar edible powders.

Tablets are prepared by direct compression, by wet granulation, or by dry granulation. Their formulations usually incorporate diluents, binders, lubricants and disintegrators as well as the compound or compounds. Typical diluents include, for example, various types of starch, lactose, mannitol, kaolin, calcium phosphate or sulfate, inorganic salts such as sodium chloride and powdered sugar. Powdered cellulose derivatives are also useful. Typical tablet binders are substances such as starch, gelatin and sugars such as lactose, fructose, glucose and the like. Natural and synthetic gums are also convenient, including acacia, alginates, methylcellulose,

polyvinylpyrrolidine and the like. Polyethylene glycol, ethylcellulose and waxes can also serve as binders.

A lubricant is generally necessary in a tablet formulation to prevent the tablet and punches from sticking in the die. The lubricant is chosen from such slippery solids as talc, magnesium and calcium stearate, stearic acid and hydrogenated vegetable oils.

Tablet disintegrators are substances that swell when wetted to break up the tablet and release the compound or compounds. They include starches, clays, celluloses, algins and gums, more particularly, corn and potato starches, methylcellulose, agar, bentonite, wood cellulose, powdered natural sponge, cation-exchange resins, alginic acid, guar gum, citrus pulp and carboxymethylcellulose, for example, may be used as well as sodium lauryl sulfate.

Tablets are often coated with sugar as a flavor and sealant, or with film-forming protecting agents to modify the dissolution properties of the tablet. The compounds may also be formulated as chewable tablets, by using relatively large amounts of pleasant-tasting substances such as mannitol in the formulation, as is now well-established in the art.

When it is desired to administer a compound as a suppository, the typical bases may be used. Cocoa butter is a traditional suppository base, that may be modified by addition of waxes to raise its melting point slightly. Water-miscible suppository bases comprising, particularly, polyethylene glycols of various molecular weights are in wide use.

The effect of the compounds may be delayed or prolonged by proper formulation. For example, a slowly soluble pellet of the compound may be prepared and incorporated in a tablet or capsule. The technique may be improved by making pellets of several different dissolution rates and filling capsules with a mixture of the pellets. Tablets or capsules may be coated with a film that resists dissolution for a predictable period of time. Even the parenteral preparations may be made long-acting by dissolving or suspending the compound or compounds in oily or emulsified vehicles that allow dispersion slowly in the serum.

The combinations of this invention may be administered in a controlled release formulation such as a slow release or a fast release formulation. Such controlled release formulations of the combination of this invention may be prepared using methods well known to those skilled in the art. The method of administration will be determined by the attendant physician or other person skilled in the art after an evaluation of the subject's condition and requirements.

The term "prodrug" means compounds that are transformed *in vivo* to yield an antiviral compound of the present invention. The transformation may occur by various mechanisms, such as through hydrolysis in blood. A good discussion of the use of prodrugs is provided by T. Higuchi and W. Stella, "Pro-drugs as Novel Delivery Systems," Vol. 14 of the A.C.S. Symposium Series, and in Bioreversible Carriers in Drug Design, ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987. The term, "prodrug" also

encompasses mutual prodrugs in which one or more antiviral compounds are combined in a single molecule that may then undergo transformation to yield the individual antiviral compounds of the present invention.

For example, if an antiviral compound of the present invention contains a carboxylic acid functional group, a prodrug can comprise an ester formed by the replacement of the hydrogen atom of the acid group with a group such as $(C_1$-$C_8)$alkyl, $(C_2$-$C_{12})$alkanoyloxymethyl, 1-(alkanoyloxy)ethyl having from 4 to 9 carbon atoms, 1-methyl-1-(alkanoyloxy)-ethyl having from 5 to 10 carbon atoms, alkoxycarbonyloxymethyl having from 3 to 6 carbon atoms, 1-(alkoxycarbonyloxy)ethyl having from 4 to 7 carbon atoms, 1-methyl-1-(alkoxycarbonyloxy)ethyl having from 5 to 8 carbon atoms, N-(alkoxycarbonyl)aminomethyl having from 3 to 9 carbon atoms, 1-(N-(alkoxycarbonyl)amino)ethyl having from 4 to 10 carbon atoms, 3-phthalidyl, 4-crotonolactonyl, gamma-butyrolacton-4-yl, di-N,N-$(C_1$-$C_2)$alkylamino$(C_2$-$C_3)$alkyl (such as β-dimethylaminoethyl), carbamoyl-$(C_1$-$C_2)$alkyl, N,N-di$(C_1$-$C_2)$alkylcarbamoyl-$(C_1$-$C_2)$alkyl and piperidino-, pyrrolidino- or morpholino$(C_2$-$C_3)$alkyl.

Similarly, if an antiviral compound of the present invention comprises an alcohol functional group, a prodrug can be formed by the replacement of the hydrogen atom of the alcohol group with a group such as $(C_1$-$C_6)$alkanoyloxymethyl, 1-$((C_1$-$C_6)$alkanoyloxy)ethyl, 1-methyl-1-$((C_1$-$C_6)$alkanoyloxy)ethyl, $(C_1$-$C_6)$alkoxycarbonyloxymethyl, N-$(C_1$-$C_6)$alkoxycarbonylaminomethyl, succinoyl, $(C_1$-$C_6)$alkanoyl, α-amino$(C_1$-$C_4)$alkanoyl, arylacyl and α -aminoacyl, or α-aminoacyl-α-aminoacyl, where each α-aminoacyl group is independently selected from the naturally occurring L-amino acids, $P(O)(OH)_2$, $-P(O)(O(C_1$-$C_6)$alkyl$)_2$ or glycosyl (the radical resulting from the removal of a hydroxyl group of the hemiacetal form of a carbohydrate).

If an antiviral compound of the present invention comprises an amine functional group, a prodrug can be formed by the replacement of a hydrogen atom in the amine group with a group such as R^X-carbonyl, R^XO-carbonyl, $NR^XR^{X'}$-carbonyl where R^X and $R^{X'}$ are each independently $((C_1$-$C_{10})$alkyl, $(C_3$-$C_7)$cycloalkyl, benzyl, or R^X-carbonyl is a natural α -aminoacyl or natural α-aminoacyl-natural α-aminoacyl, $-C(OH)C(O)OY^X$ wherein (Y^X is H, $(C_1$-$C_6)$alkyl or benzyl), $-C(OY^{X0})\ Y^{X1}$ wherein Y^{X0} is $(C_1$-$C_4)$ alkyl and Y^{X1} is $((C_1$-$C_6)$alkyl, carboxy$(C_1$-$C_6)$alkyl, amino$(C_1$-$C_4)$alkyl or mono-N- or di-N,N-$(C_1$-$C_6)$alkylaminoalkyl, $-C(Y^{X2})\ Y^{X3}$ wherein Y^{X2} is H or methyl and Y^{X3} is mono-N- or di-N,N-$(C_1$-$C_6)$alkylamino, morpholino, piperidin-1-yl or pyrrolidin-1-yl.

The compositions for use in accordance with the present invention may be formulated in a conventional manner using one or more physiologically acceptable carriers or excipients. Antiviral, SMIP, SMIS, or other immunomodulating compounds are prepared or obtained as described herein and in the US Patents and published international patent applications listed in

Table 1, Table 2, Table 34 and Table 35. The antiviral compounds can be formulated in pharmaceutically acceptable compositions suitable for delivery to the lungs. Particular formulations include dry powders, liquid solutions or suspensions suitable for nebulization and propellant formulations suitable for use in metered dose inhalers. The preparation of such formulations is well know to those skilled in the art, and is described in US Patent Nos. 5,814,607 and 5,654,007 and in the US Patents and published international patent applications listed in Table 3 the disclosures of which are incorporated herein by reference.

Dry powder formulations will comprise an antiviral compound in a dry, optionally lyophilized form with a particle size within a preferred range for deposition within the lung. Typically the particle size for deposition in the lung will range between 1 and 5 μm. When systemic delivery of the antiviral compound via absorption from the lung into the bloodstream is desired the antiviral compound formulation particle size is generally between 0.1 and 2 μm in size. The preferred size range of particles can be produced using methods such as jet-milling, spray drying and solvent precipitation, for example. Dry powder devices typically require a powder mass in the range from about 1 mg to 100 mg to produce an aerosolized dose. Thus, the antiviral compound will typically be combined with a pharmaceutically acceptable dry bulking powder. Preferred dry bulking powders include sucrose, lactose, trehalose, human serum albumin (HSA), phospholipids and glycine as well as those disclosed in the documents listed in Table 3. Dry powders can be administered to the subject in conventional dry powder inhalers. For liquid formulations the antiviral compound can be dissolved in any recognized physiologically acceptable carrier for use in delivery of aerosolized formulations. Such carriers include buffered and unbuffered aqueous solutions for water soluble compounds, and physiological solutions including saline solution (preferably between 0.2 and 2 N NaCl). For antiviral compounds with limited solubility, other liquid vehicles such as ethanol, propylene glycol and ethanol-propylene combinations may be used. The antiviral compounds may also be administered as solids in suspension.

For administration by inhalation, the compositions for use according to the present invention are conveniently delivered in the form of an aerosol spray administered via pressurized packs or a nebulizer, with the use of a propellant, *e.g.*, air, dichlorordifluoromethane, dichloroterafluoroethane or other suitable gas. Preferably, for incorporation into the aerosol propellant, the antiviral compound formulations of the present invention will be processed into respirable particles as described above for the dry powder formulations. The particles are then suspended in the propellant, optionally being coated with a surfactant to enhance their disbursement. In the use of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount.

Commercially available jet nebulizers are available and may be used to deliver aerosolized antiviral compound to a subject. Such jet nebulizers include, but are not limited to, those supplied by AeroTech 11 (CIS-US, Bedford, Mass.). In addition, for delivery of aerosolized antiviral compound to the lungs of a subject an oxygen source can be attached to the nebulizer providing a flow rate of, for example, 10 L/min. In general, inhalation is performed over a 5-40 minute time interval through a mouthpiece during spontaneous respiration. The present invention provides for novel compositions comprising a suitable carrier and aerosolized antiviral compound in doses sufficient to reduce or ameliorate viral load and SARS symptoms in subjects having SARS. Such doses can be lower than corresponding systemic doses that may be used to those generally used to reduce or ameliorate viral load and SARS symptoms in subjects having SARS.

The antiviral, SMIP, SMIS, and immunomodulating compositions of the present invention may be administered with a steroidal anti-inflammatory drug for the treatment of SARS and SARS symptoms. Examples of steroidal anti-inflammatory drugs of the invention include hydrocortisone, prednisolone, dexamethasone, triamcinolone acetonide, fluocinolone acetonide, fludrocortisone acetate, betamethasone, *etc.*

The antiviral compound composition of the invention is nebulized predominantly into particle sizes allowing a delivery of the drug into the terminal and respiratory bronchioles. For efficacious delivery of antiviral compound to the lung endobronchial space of airways in an aerosol, the formation of aerosol particles having mass medium average diameter predominantly between 1 to 5 μm is necessary. The formulation must additionally provide conditions that would not adversely affect the functionality of the airways. Consequently, the formulation must contain enough of the drug formulated under the conditions that allow its efficacious delivery while avoiding undesirable reaction.

For liquid solutions and suspensions, the choice of the nebulizer is made from among commercially available nebulizers. The jet nebulizers known as Sidestream O, obtained from Medicaid and Pari LCS, LC Plus, and eFlow obtained from Pari Respiratory Equipment, Richmond, Virginia, are examples of typical nebulizers suitable for the practice of the invention. Ultrasonic nebulizers that produce appropriate particle sizes of about 1 to 5 μm such as Aerosonic by DeVilbiss and UltraAire by Omron are also suitable.

Advantageously, the present invention also provides for a kit for use by a consumer for the treatment and/or prevention of SARS. Such a kit comprises: (a) a pharmaceutical composition comprising a therapeutically effective amount of at least one compound from among those described herein or listed in Table 34 and Table 35 or described in the US Patents and published international patent applications listed in Table 1, Table 2, and Table 35 and a pharmaceutically acceptable carrier, vehicle or diluent; (b) a container for holding the pharmaceutical composition;

and, optionally, (c) instructions describing a method of using the pharmaceutical compositions for the treatment and or the prevention of SARS. The kit may optionally contain a plurality of antiviral compounds for the treatment of SARS wherein the anti viral compounds are selected from 3C-like protease inhibitors and papain-like protease inhibitors. In a further embodiment, the kit contains an antiviral compound which is an RNA-dependent RNA polymerase inhibitor. When the kit comprises more than one antiviral compound, the antiviral compounds contained in the kit may be optionally combined in the same pharmaceutical composition.

A "kit" as used in the instant application includes a container for containing the separate compositions such as a divided bottle or a divided foil packet. The container can be in any conventional shape or form as known in the art that is made of a pharmaceutically acceptable material, for example a paper or cardboard box, a glass or plastic bottle or jar, a re-sealable bag (for example, to hold a "refill" of tablets for placement into a different container), or a blister pack with individual doses for pressing out of the pack according to a therapeutic schedule. The container employed can depend on the exact dosage form involved, for example a conventional cardboard box would not generally be used to hold a liquid suspension. It is feasible that more than one container can be used together in a single package to market a single dosage form. For example, tablets may be contained in a bottle that is in turn contained within a box.

An example of such a kit is a so-called blister pack. Blister packs are well known in the packaging industry and are being widely used for the packaging of pharmaceutical unit dosage forms (tablets, capsules, and the like). Blister packs generally consist of a sheet of relatively stiff material covered with a foil of a preferably transparent plastic material. During the packaging process, recesses are formed in the plastic foil. The recesses have the size and shape of individual tablets or capsules to be packed or may have the size and shape to accommodate multiple tablets and/or capsules to be packed. Next, the tablets or capsules are placed in the recesses accordingly and the sheet of relatively stiff material is sealed against the plastic foil at the face of the foil that is opposite from the direction in which the recesses were formed. As a result, the tablets or capsules are individually sealed or collectively sealed, as desired, in the recesses between the plastic foil and the sheet. Preferably the strength of the sheet is such that the tablets or capsules can be removed from the blister pack by manually applying pressure on the recesses whereby an opening is formed in the sheet at the place of the recess. The tablet or capsule can then be removed via said opening.

It maybe desirable to provide a written memory aid, where the written memory aid is of the type containing information and/or instructions for the physician, pharmacist or subject, *e.g.*, in the form of numbers next to the tablets or capsules whereby the numbers correspond with the days of the regimen which the tablets or capsules so specified should be ingested or a card that contains the same type of information. Another example of such a memory aid is a calendar

printed on the card *e.g.*, as follows "First Week, Monday, Tuesday," . . . *etc* . . . . "Second Week, Monday, Tuesday, . . ." *etc.* Other variations of memory aids will be readily apparent. A "daily dose" can be a single tablet or capsule or several tablets or capsules to be taken on a given day. Also a daily dose of one or more component(s) of the kit can consist of one tablet or capsule while a daily dose of another one or more component(s) of the kit can consist of several tablets or capsules.

Another specific embodiment of a kit is a dispenser designed to dispense the daily doses one at a time in the order of their intended use. Preferably, the dispenser is equipped with a memory-aid, so as to further facilitate compliance with the regimen. An example of such a memory-aid is a mechanical counter that indicates the number of daily doses that has been dispensed. Another example of such a memory-aid is a battery-powered micro-chip memory coupled with a liquid crystal readout, or audible reminder signal which, for example, reads out the date that the last daily dose has been taken and/or reminds one when the next dose is to be taken.

EXAMPLES

Example 1-EXAMPLE of a SARS VIRUS ISOLATE

A SARS virus was isolated from clinical specimens of a patient in Frankfurt, Germany (FRA). The isolate was grown in Vero cells. RNA of the SARS virus was extracted and amplified by RT-PCR. Nucleotide sequence of the viral genome was determined by direct sequencing of the PCR product. Computer analysis was used to predict the features of the genome, to compare it to previously known coronaviruses and to the sequence of different SARS virus isolates.

More specifically, isolation and sequence was performed as follows. After the third passage of the SARS virus in Vero cells, viral particles were purified by ultra centrifugation from $3x10^7$ cells supernatant. Viral RNA was extracted by Triazol method (Gibco-BRL). Viral RNA (200 ng) was transcribed into cDNA with avian RNaseH- thermostable reverse transcriptase following the instructions of the manufacturer (ThermoScript RT System, Invitrogen). Briefly, either 50 pmoles of oligo $(dT)_{20}$ (SEQ ID NO: 7389) or 25 ng of random hexamers were used to prime the RT reaction in a 20 μl final volume. Amplification and sequencing of the SARS genome were accomplished by direct sequencing of PCR products obtained with: i) specific primers from conserved regions of homology found through multiple alignment among known coronaviruses; ii) oligonucleotides designed around short sequences of SARS isolates available on the Web through WHO network laboratories; iii) degenerate primers to amplify the cDNA mixture with multiple overlapping fragments as end products. Gap closure

was realized by long distance PCR with high fidelity Taq (Expand High Fidelity system, Roche) using primers designed on selected fragments. Sequence was collected by primer walking using a BigDye terminator chemistry (Applied Biosystems) and an automated DNA sequencer (3700 capillary model, Applied Biosystems). After obtaining a first pass of the entire genome, a set of both forward and reverse primers were used to amplify and sequence *de novo* the genome using as a template DNA segments of 2 kb on average. Readings from overlapping fragments were automatically assembled by AutoAssembler (Applied Biosystems) and the 29,740 bp contiguous edited manually.

Computer analysis of the sequence was performed as follows. The GCG Wisconsin Package suite (version 10.0) was used for computer analysis of gene and protein sequences. The PSORT program (*http://psort.nibb.ac.jp/*) was used for localization predictions. For secondary structure analysis, the PHD software available on the Web at *http://cubic.bioc.columbia.edu/predictprotein/* was applied. The PSI-BLAST algorithm was used for homology searches (*http://www.ncbi.nlm.nih.gov/blast*) using the non-redundant protein database. ClustalW was applied to obtain multiple sequence alignments of gene and protein sequences. The LearnCoil-VMF program was used to predict coiled-coil regions in the spike proteins (*http://learncoil-vmf.lcs.mit.edu/cgi-bin/vmf*). Leucine zippers were predicted with the program 2ZIP, available at *http://2Zip.molgen.mpg.de*.

Phylogenetic analysis was performed using the neighbor-joining algorithm as implemented in the program NEIGHBOR within the Phylogeny Inference Package (Phylip) (Felsenstein J 1993, program distributed by the author). Bootstrap analysis was always performed with 100 replicates using the program Seqboot. Trees were handled and displayed using TreeView. The program HMMER was used to generate sequence profiles from multiple sequence alignments of the S1 domains of spike proteins. Subsequently, the HMMPFAM program was used to compare the S1 domain of SARS spike to the profiles.

The genome of this SARS virus isolate is 29,740 bases long and the overall structure of the genome is similar to that of the three known groups of coronaviruses. Starting from the 5' end a leader sequence, an untranslated region (UTR) and two overlapping open reading frames coding for one polyprotein containing the enzymes necessary for replication can be identified. They are followed by a region coding for the spike (S), envelope (E), matrix (M), nucleocapsid (N) structural proteins and eight additional ORFs specific for the SARS virus. At the 3'-end of the genome a UTR with a poly(A) is located. The overall homology to coronaviruses groups 1, 2 and 3 is low and therefore the SARS virus belongs to a new group (group 4) of coronavirus. More detailed analysis of the spike protein amino acid sequence shows that the SARS virus isolate is more closely related to coronavirus group 2.

The complete genome sequence of the SARS virus isolate is 29,740 bp in length. The sequence is available on Genbank and has a GC content of 40.8%, comparable with that of known viruses of the same family. Genome structure is similar to that of other coronaviruses. 14 open reading frames have been predicted. The principal features of the genome and gene products are illustrated reported in Figure 17 and Table 10. The comparison between the SARS genome and those of group 1, 2 and 3 coronaviruses is reported in Figure 18.

Nucleotides 1-73 contain a predicted RNA leader sequence followed by an untranslated region (UTR) of 197 nucleotides. The UTR is followed by two overlapping open reading frames (ORF1a, ORF1b), which encompass two-thirds of the genome (nucleotides 265-21485). They encode for a large polyprotein, which is predicted to be processed by viral proteases to generate the replicase complex. The 3' part of the genome contains the genes coding for the four structural proteins (S, spike protein, E, envelope protein, M, matrix glycoprotein, and N, nucleocapsid protein), and eight predicted ORFs of unknown function (Figure 17). Finally, at the 3' end of the genome, we found a second UTR of 340 bases followed by a poly(A) tract. We identified a putative intergenic (IG) sequence also referred to as transcription-associated sequence (TAS), which is a typical feature for coronaviruses. The IG sequence is characterized by 6-18 nucleotides present at the 3' end of the leader and can be found in front of each gene. The IG sequence plays a key role in RNA transcription and its regulation. The IG sequence of the SARS virus is characterized by the sequence SEQ ID NO: 7293 and is present nine times in the genome (Figure 17). The sequence of the leader and IG are peculiar for each coronavirus and represent a specific signature for the virus.

The Replicase Region

The replicase gene, ORF1ab (SEQ ID NO: 7232), consists of two overlapping ORFs, ORF1a and ORF1b, which can be translated as a single polyprotein by frame shift of the ribosome in position 13,393, within the polymerase encoding region. See Brierley et al, *Embo J* 1987: 6(12): 3779-3785. As expected, a stem-loop sequence is present ten base pairs downstream of this site (SEQ ID NO: 7390; 5'-CGGTGTAAGTGCAGCCCGTCTTACACCG-3'). The polyprotein is cleaved co- and/or post-translationally into multiple proteins by its own encoded proteases. Using the cleavage consensus sequence and by analogy with other coronaviruses, we have mapped the possible cleavage sites of the polyprotein and have identified 14 products, which comprise the leader protein p28, the homologue of the MHV p65 protein and other twelve proteins, named from nsp1 to nsp13 (nsp, non structural protein) (Figure 17 and Table 10). The amino acid sequence analysis suggests the presence of several functional motifs within the putative ORF1ab proteins. In particular, we have mapped two potential proteases (nsp1 and nsp2), one growth factor-like motif (nsp7) within ORF1a, whereas in ORF1b we identified the RNA polymerase (nsp9), and a

predicted helicase (nsp10). The other predicted cleavage products (nsp3, nsp4, nsp5, nsp6, nsp11, nsp12 and nsp13) are proteins of unknown function. Many of these proteins are presumably present in the RNA replication complex, which is associated with the membranous structures in the infected cells. In particular, nsp3 and nsp4 contain hydrophobic domains. As shown in Figure 18, the replicase region of SARS has a similar organization to group 1, 2 and 3 coronaviruses; however, the overall aminoacid conservation is low (Table 11). The most conserved proteins are the polymerase and the helices.

Nsp1 is the papain-like cysteine protease (PLP), which cleaves the first two protein products (leader protein p28 and p65 homologue). Within the nsp1 of MHV, two domains with papain-like protease activity (PLP1 and PLP2) have been mapped, (Kanjanahaluethai *et al* (2000) *J. Virol* 74(17):7911-21) which are also conserved with Bovine, transmittable gastroenteritis virus (TGV) and Human 229E coronaviruses. However, by sequence alignment with the SARS nsp1, we identified only one PLP domain containing the catalytic residues Cys833 and His994.

Nsp2 is the chymotrypsin-picornavirus 3C-like protease (3CLp), which is responsible for the post-translational processing of the other 12 proteins, most of them cleaved at Q/A or Q/S sites. (Ziebuhr *et al* (1999) *J. Virol* 73(1):177-85). It also performs autoproteolytic activity. The principal catalytic residues are well conserved with other coronaviruses and are located at position His41 and Cys145. Furthermore, even the conserved aminoacids Tyr161 and His163, which are believed to be involved in substrate recognition and to be indispensable for proteolytic activity, (Hegyi *et al* (2002) *J. Gen Virol* 83(Pt3):581-593) were found in the sequence of the SARS 3CLp.

The invention includes the orf1ab sequence of SEQ ID NO: 9960 and the orf1a sequence of SEQ ID NO: 9961, including fragments, variants, homologs, *etc.* thereof.

The Structural Region

Analysis of the nucleotide sequence at the 3' part of the SARS genome identified 12 predicted open reading frames. They are coded within 8.2 kb and comprise the four structural proteins S, E, M and N, common for all coronaviruses and eight predicted ORFs, which are specific for this virus (Figure 18). SARS-specific IG sequences upstream of most ORFs (Figures 17 & 18) suggest that most genes are likely to be transcribed independently. Interestingly, sequences identical to the group 2 IG are also present at the end of the RNA leader and in front of the Matrix encoding gene and of ORF 10.

The spike is a type I glycoprotein, which forms the large spikes on the surface of the virion and is responsible for receptor-binding and membrane fusion. (Gallagher (2001) *Adv Exp Med Biol* 494: 183-92). The protein is 1255 residues long with 17 predicted N-glycosylation sites. It has a 13aa leader peptide and a 17 aa C-terminal membrane anchoring sequence (1202-1218).

Some (MHV, HCoV-OC43, AIBV and BCoV), but not all (TGV, FIPV, HCoV-229E) coronavirus spike proteins are proteolytically cleaved in two subunits, S1 and S2. S1 is supposed to form the bulbous head, which stays non-covalently linked to the C-terminal membrane anchor. Cleavage is mediated by a basic aminoacid sequence, which resembles the consensus sequence for a furin cleavage site. (Garten *et al.*, *Biochimie* 1994; 76(3-4): 217-225). However, in case of this SARS virus isolate, we were not able to identify such a sequence, implicating that the S protein of this SARS virus isolate is unlikely to be cleaved during maturation. Secondary structure predictions indicated that the global architecture of the spike protein is conserved within all known coronaviruses. The S1 domain is mainly formed by beta sheets and likely adopts a globular fold, while in the S2 domain extensive alpha helical regions are predicted. In addition, the LearnCoil-VMF program, specifically designed to identify coiled-coil-like regions in viral membrane-fusion proteins, predicts two coiled-coils within S2, spanning aminoacids 900-1005 and 1151-1185, respectively (Figure 19). Both coiled-coil regions contain a leucine-zipper motif, which is also present in the spikes of all coronaviruses. Leucine zippers are known to promote protein oligomerization; since the spike proteins of TGV and MHV form hetero-trimers, (Delmas *et al*, *J Virol* 1990; 64(11):5367-5375) (Godeke, *et al.*, J *Virology* 2000; 74(3):1566-1571) it is conceivable that in SARS leucine zippers play a role in promoting and/or stabilizing a similar quaternary structure. The spike protein plays a major role in the biology of coronaviruses because the S1 domain contains the receptor-binding domain and the virus neutralizing epitopes, while the S2 domain is involved in the process of membrane fusion, which is essential for virus infectivity. As expected, multiple sequence alignment of different spike proteins showed a major degree of variability within the S1 domain, whereas S2 is more conserved.

The envelope protein E is a very short polypeptide of 76 aa, involved in the morphogenesis of the virion envelope. (Godet *et al.*, *Virology* 1992; 188(2):666-675). Computer analysis predicts a long transmembrane domain close to the N-terminus and two N-glycosylation sites. The level of aminoacid similarity with other coronaviruses is very low and the best homology is with the small envelope protein of the transmissible gastroenteritis virus (TGV).

The matrix glycoprotein (M) is a 221-residue polypeptide with a predicted molecular weight of 25 kDa. Computer analysis predicts a topology consisting of a short aminoterminal ectodomain, three transmembrane segments and a carboxyl terminus located at the interior side of the viral envelope. In analogy with the matrix glycoprotein of TGV, that of the avian infective bronchitis virus (AIBV) and that of the hypervirulent MHV-2 strain the SARS M glycoprotein is N-glycosylated at the N-terminus. SARS M protein shows highest similarity to group 2 viruses (Table 11).

Finally, the nucleocapsid protein N is a 397-residue-long phosphoprotein that interacts with viral genomic RNA to form the nucleocapsid. The level of conservation with other coronaviruses is low, ranging from 26,9% identity with the HCoV-229E to 37,4% identity to the Bovine coronavirus (BcoV) (Table 11). Epitope analysis of the nucleocapsid protein has been carried out (Li *et al.* (2003) *Geno Prot & Bioinfo* 1:198-206) in which the epitope site at the C terminus of the protein was located as SEQ ID NO: 7394 (amino acids 371-407 of SEQ ID NO: 6052).

In addition to the above fundamental proteins, many viruses express a set of other peptides, which are generally dispensable for viability, but can influence the infectivity potential of the virus. (de Haan *et al.*, Virology 2002; 296(1):177-189). These proteins are generally conserved within members of the same serogroup, but differ profoundly among the groups. For this reason, they are generally referred to as group-specific proteins (Figure 11). Members of the group 1, represented here by HcoV-229E, have two group-specific genes located between the S and E genes and sometimes one or two ORFs downstream of the N gene, preceding the 3' UTR region of the genome. Viruses of the group 2, with MHV as prototype, have two group-specific genes (2a and HE) between ORF1b and S, as well as other two between S and E genes. Finally, the group 3 viruses, represented by the prototype AIBV, have two group-specific genes between S and E and other two between the M and N genes.

With the exception of the hemagglutinin esterase HE, for which hemagglutinating and acetyl-esterase enzymatic activities have been demonstrated, all the other group-specific ORFs encode proteins whose role has not yet been established.

Interestingly, the arrangement of specific genes in the SARS genome is peculiar and the predicted ORFs do not display any significant homology with ORFs present in the other coronaviruses, nor with any other known protein from different organisms. Like viruses of the group 1 and 3, SARS lacks the HE hemagglutinin and does not contain ORFs between the ORF1b and the S gene. Furthermore, two predicted ORFs (ORF3 and ORF4) are encoded in the region between S and E, and superimpose for most of their length. ORF3 has an IG sequence 2 bp upstream of the ATG start codon. In contrast to the other groups, SARS contains five predicted ORFs in the region between M and N genes. ORF7 is located 10 bases downstream of the stop codon of M gene, and has an IG sequence 155 nucleotides upstream from the ATG start codon. Similarly, ORF8 and ORF10 present an IG right upstream of their ATG start codons. On the other hand, the 5' ends of ORF9 and ORF11 shortly superimpose with the flanking genes, and for this reason they do not need an IG to activate transcription. ORF12 totally superimposes with the N gene and shares very low homology with a 22kDa protein of the MHV virus, coded in the corresponding region.

Despite the absence of indications of possible localization and function deriving from sequence similarity, ORF3, ORF7 and ORF8 contain hydrophobic segments, suggesting association with membrane structures. In addition, ORF3, the longest among the SARS specific gene, is the only one that encodes for a peptide containing a high number of predicted O-glycosylation sites (Table 11). Predicted N-glycosylation sites have been identified in ORF3, ORF11 and ORF12.

Two shorter ORFs in the non-structural regions are SEQ ID NOS: 9965 and 9966. The invention includes polypeptides with these sequences, and also fragments, variants, *etc.*

Phylogenetic analysis

The substitution frequency within 922 conserved bases from the *pol* gene of eleven coronaviruses from the three different serogroups has been used in the past to show that the variability within members of each serogroup is much smaller than between members of different serogroups, confirming the previously described serological groupings. (Stephensen *et al.*, Virus Res 1999; 60(2):181-9). We used the 922 bp region of the *pol* gene of SARS and aligned it with the same fragment from other 12 coronaviruses. The tree obtained showed that the SARS virus is distinct from the other three groups of coronaviruses (Figure 20). Similar results were obtained using the full-length aminoacid sequences of *pol,* 3CL-protease and helicase from the replicase region and those of the spike and the matrix glycoproteins from the structural region (data not shown). These data confirmed that the entire genome of the SARS virus clusters in a new group (group 4) of coronavirus.

To gain more resolution for possible evolutionary relationships we performed the analysis using consensus sequences of predicted domains of the proteins. In particular, we generated consensus sequences of the S1 domain of the spike protein from the group 1 and group 2 and then we compared them to the S1 domain of the SARS spike. No consensus could be generated from the group 3 since only the spike protein of AIBV is known. Interestingly, the tree constructed from the alignment of SARS S1 with the consensus generated from the two groups of spike proteins was different from that in Figure 20, and showed a much closer relationship between SARS and group 2 coronaviruses (Figure 21A). Further analysis showed that 19 out of the 20 cysteines present in the SARS S1 domain are spatially conserved with the group 2 consensus sequence, while only five are maintained either within the group 1 and group 3 sequences (Figure 21B). Given the fundamental role played by cysteines in protein folding, it is likely that the S1 domain of SARS and group 2 coronaviruses share a similar spatial organization.

Sequence variability between SARS coronaviruses

We compared the FRA sequence to the four complete SARS genomes available on the Web. A total of 30 mutations were detected. Nine of these mutations were silent while 21 resulted in aminoacid substitutions (Table 12). Within ORF1a, three silent and seven productive mutations were detected. In ORF1b, there were five silent and three productive mutations. One of the productive mutations was caused by two nucleotide substitutions resulting in a single aminoacid change. Five changes were located in the spike protein, four of these were productive and one silent. Two productive mutations were in ORF3 and in the matrix glycoprotein M. One productive mutation each was in ORF10 and in the nucleocapsid protein N.

The overall difference between FRA and TOR2 was of nine nucleotides resulting in two silent mutations and seven aminoacid changes. The difference between FRA and Urbani is 12 nucleotides, which result in five silent mutations and seven aminoacid changes. For CUHK 16 nucleotides were different, five of which were silent mutations. For FRA and HKU 14 nucleotide changes resulted in four silent and nine productive mutations.

EXAMPLE 2 -Production, Inactivation and Purification of Whole SARS Virus Using MCS Chromatography Resin Purification Followed by Density Gradient Ultracentrifugation

A SARS isolate FRA1 (EMBL: AY310120) was passaged on VERO cells that were cultivated in DMEM (Gibco: Cat No. 21969-035, Lot No. 3078864), Penicillin/Strep (Gibco: Cat No. 15070-063, Lot No. 1120042), and 3% FCS (Gibco: Cat No. 10270-106, Lot No. 40F6130K) at 37°C, 5% CO_2. Trypsin (Gibco: Cat No. 25300-054, Lot No. 3078729) was used for detaching the cells.

For virus production the third passage was used for inoculation of VERO cells at a moi of ~0.1. Cells were incubated with the virus for 1 h at 37°C in infection medium (DMEM without PS, FCS); after 1h cells were washed twice and further incubated at 37°C for 48 h in the presents of 3% FCS and antibiotics. The supernatant was harvested 48 hours post infection (p.i.) and precleared by centrifugation at 3000 rpm at 4°C for 10 min.

The SARS virus was inactivated by β-propiolactone (BPL) treatment (1:2000) for 18 h at 4°C, followed by 3 h at 37°C. Testing the virus on successful inactivation, VERO cells were incubated with 10 ml BPL treated supernatant for 4 days at 37°C; subsequentially, the supernatant was transferred to a fresh VERO cell culture and further incubated for another 4 days. Cells were checked for cytopathic effect (CPE).

200 ml of the BPL-inactivated SARS virus harvest was then clarified using a 0.65 μm-pore-size filter (47 mm diameter) to pass virus particles and retain cell debris. The filter unit was connected to a Masterflex pump, which accomplished a consistent flow rate of 40 ml/min.

A. MCS Chromatography Purification Step

The filtered virus suspension was then subjected to MCS chromatography. The MCS column was prepared as follows. 27 ml slurry led to 14 ml sedimentated resin which was packed using a Götec Superformance Column (diameter 1.0 cm, height 15.7 cm, volume 12.33 ml). 1% of the column volume of a 1% acetone solution was injected to the column and the column was run with a flow of 100 cm/h. The HETP, N and A_s values were then calculated as HETP: 0,056 cm, N / m: 1790 and $A_s = 1.20$.

The amount of proteins in the purified solution after the MCS chromatography step were assessed with a bicinchoninic acid (BCA) method (Interchim) (*see, e.g.*, *http://www.piercenet.com/files/bca.pdf*) and electrophoresis.

SDS-PAGE was done in accordance to Laemmli, *Nature* (1970) 227:680-685. Samples for SDS-PAGE were diluted to a protein concentration of 77 μg/ml. Different protein concentrations were loaded depending on the gel types used (10/12/15 Wells, Novex/Invitrogen):

Number of Wells	Protein Concentration in the Dilution	Load	Protein/Well
10 Wells	77 μg/ml	20 μl	1 μg
12 Wells	77 μg/ml	15-20 μl	0.75 - 1 μg
15 Wells	77 μg/ml	10 μl	0.5 μg

Samples for use in a reducing SDS-PAGE were prepared as follows:

	26 μl sample or diluted sample
	+ 10 μl NuPage Sample Buffer (4x) SDS NP0003
	+ 4 μl TCEP Bondbreaker Solution 77720 (1:2 in MilliQ water)
Final Volume:	40 μl

The samples were heated for 10 minutes at 70°C or left at room temperature for 1 hour (leaving the samples at room temperature prevents the M protein of Corona Virus to coagulate/forming complexes), and then centrifuged for approximately one minute at 14,000 rpm in a table top centrifuge.

Markers for use on the gel were prepared as follows. Gel bands containing less than 1 μg of proteins were easily visualised with the silver staining procedure using the Silver Staining Kit Protein, Plus One Staining Protocol (Pharmacia Biotech).

Western blotting was performed as follows. A semi-dry blotting technique was used to transfer the proteins from the SDS gel to a nitrocellulose membrane. The transfer was performed with a current of 0.8 mA/cm^2 for 1 hour. A rabbit polyclonal antibody against SARS virus was used to perform the immuno probing using the Western Breeze, Novex Chromogenic Western Blot Immunodetection Kit (Novex/Invitrogen).

The chromatogram of the inactivated SARS MCS capture step is depicted in FIGURE 27. To estimate purity, MCS chromatography fractions were analysed by silver staining on NuPage

10% or 4-12% Bis-Tris-Gel (Novex) under reduced conditions, heated for 10 minutes at 70°C (Figure 28). The fractions were also analysed under the same conditions by western blot (Figure 29) to estimate purity, using PAK 11/03 SARS Cov 270603 neutralizing titer 1:512 (this antibody was used for this and subsequent western blots). Purity estimates are as follows:

Sample	Volume / ml	[Protein] / µg/ml	Total Protein / mg	Step Recovery Protein / %
Corona Harvest	100	2547.6	254.76	100
After Filtration = Load	100	2440.3	244.03	95.8
Flow Through	85	2321.4	197.32	**77.5**
Wash	49.32	468.5	23.11	9.1
Peak 1	12.12	252.7	3.062	**1.2**
Total Recovery	-	-	464.4	86.5

B. Density Gradient Ultracentrifugation Step

The eluted SARS virus fraction was then subjected to density gradient ultracentrifugation with a swinging bucket rotor to further purify the inactivated virus. 3 ml of MCS peak fraction were loaded onto a linear gradient (15-60% sucrose; 17 ml 15% and 17 ml 60% sucrose in gradient mixer). The separation was performed with a Beckman SW 28 rotor at 20,000 rpm for 2 hours.

The content of sucrose and protein in the linear density gradient ultracentrifugation fractions are depicted in the following table, the graph in figure 30 and the estimation of purity in figure 31:

Fraction	Fraction Size / ml	[Sucrose] / %	[Protein] / µg/ml
1	2	61	96.12
2	2	59.4	98.62
3	2	57.5	87.63
4	2	54.5	86.91
5	2	50.5	79.9
6	2	47.2	74.3
7	2	43.7	68.05
8	2	40.2	60.43
9	2	37.2	57.38
10	2	34	53.12
11	2	30	50.63
12	2	25.7	35.02
13	2	22.4	35.33
14	2	19.5	39.25
15	2	15.5	69.79
16	2	8.5	169.03
17	2	8.5	128.96

The protein concentration of fraction 11 (Figure 31 SDS-gel) was measured again against a standard curve prepared in 30% sucrose and lead to a protein concentration of 3.67 µg/ml (0.05

μg on the gel). The M protein appears to be missing in this preparation possibily due to sample treatment procedure (heated samples).

There may be discrepancies in the protein concentration measurements in Table 2 due to sucrose interference with this assay.

EXAMPLE 3 -Production, Inactivation and Purification of Whole SARS Virus Using MCS Chromatography Resin Purification Followed by Density Gradient Ultracentrifugation

Inactivated SARS virus was prepared as described in Example above.

A. MCS Chromatography Purification Step

In this example, 200 ml of inactivated SARS virus harvest were subjected to MCS chromatography. The chromatogram of the capture step of inactivated SARS virus purification with MCS is depicted in FIGURE 32, the protein recovery in the following table and the estimation of purity in FIGURE 33:

Sample	Volume / ml	[Protein] / μg/ml	Total Protein / mg	Step Recovery Protein / %
Corona Virus Harvest	200	2239.2	447.83	100
After Filtration = Load	200	2245.1	449.02	100.3
Flow Through	185	2126.3	393.37	**87.8**
Wash	49.32	450.1	22.2	5.0
Peak 1	4.43	1245.6	5.52	**1.2**
Total Recovery	-		421.08	93.7

B. Density Gradient Ultracentrifugation Step

3.5 ml of MCS peak fraction were then loaded onto a linear gradient (15-40% sucrose: 16 ml 15% and 16ml 40% sucrose in gradient mixer). The separation was performed with a Beckman SW 28 rotor at 20,000 rpm for 2 hours.

The content of sucrose and protein in the linear density gradient ultracentrifugation fractions are depicted in the following table and the graph in FIGURE 34:

Tube	Fraction Size / ml	[Sucrose] / %	[Protein] / μg/ml
1	2	40	45.86
2	2	39	45.68
3	2	37.5	44.14
4	2	35.5	37.82
5	2	33.5	34.48
6	2	31.5	31.76
7	2	30.5	29.49
8	2	28	30.87
9	2	25.5	31.7
10	2	23.5	26.74
11	2	21.75	23.58
12	2	20	35.33
13	2	18	96.38
14	2	14.5	523.79

15	2	8	941.97
16	2	8	696.7

Protein recovery is shown in the following table and the estimation of purity is shown in figure 35. Electron Micrograph pictures of density gradient fractions 8, 9 and 10 are shown in figure 36:

Step	Volume / ml	Protein / μg/ml	Total Protein / mg	Step Protein %
Load	3.5 ml	1245.6	4359.6	100
Bulk Protein Fractions	3.5 ml	720.8	4324.9	99.2
Viral Peak Fraction	8 ml	29.7	237.6	**5.5**
Total Recovery			4562.5	104.7

EXAMPLE 4 - Mouse Immunization with Inactivated SARS Virus

Mice were immunized subcutaneously on days 0, 14, and 28 with 5 μg BPL-inactivated SARS-CoV particles (BPL-SARS-CoV), either alone or together with Alum or MF59 as adjuvants. Serum was collected on days 0 (pre-immunization), 13 (post 1st immunization), 28 (post 2nd), and 35 (1 week post 3rd immunization). Neutralizing antibodies were assessed for blocking SARS-CoV infection of Vero cells *in vitro*. After 3 immunizations, neutralization titers were in the range 1:100-1:1000, which are levels similar to those present in the serum of SARS convalescent patients. As shown in the following table, the non-adjuvanted vaccine induced neutralizing antibody after the third immunization, and potency of this vaccine was enhanced significantly by including the adjuvants, with neutralizing antibody appearing after then 2nd immunization and overall titers increasing after then 3rd immunization:

	Neutralization Titer			
Immunogen	**pre**	**post 1st**	**post 2nd**	**post 3rd**
BPL-SARS-CoV+MF59 (5 μg)	< 1:20	< 1:20	1:158	1:630
BPL-SARS-CoV+Alum (5 μg)	< 1:20	< 1:20	1:67	1:612
BPL-SARS-CoV (5 μg)	< 1:20	< 1:20	< 1:20	1:71
PBS	< 1:20	< 1:20	< 1:20	< 1:20

EXAMPLE 5 - Balb/cMouse Immunization with Inactivated SARS Virus

A Balb/c mouse model for SARS infection has been developed (Subbarao *et al.* (2004), *J.Virol.*, 78:3572-77. In this model, Balb/c mice are inoculated intranasally with 10^4 $TCID_{50}$ of virus. At 48 hours post-inoculation, a 2-log increase in the $TCID_{50}$ virus titer can be detected in the lungs of infected mice. While virus replication is readily detected, the mice do not show any SARS disease symptoms and spontaneously clear the virus one week after inoculation. A decrease in virus titer in previously-immunized animals as compared to control animals demonstrates a protective effect of the vaccine being evaluated.

In this example, four Balb/c mice per group are immunized three times with 5 μg BPL inactivated SARS-CoV (days 0, 14, 28) either alone or in combination with MF59 and

challenged with 10^4 $TCID_{50}$ of SARS-CoV on day 43. Two days following virus challenge the mice are euthanized and SARS-CoV is quantified from nasal turbinates (NT) and lungs and the mean virus titer for each mouse is measured. Control groups received PBS alone, or an influenza virus vaccine (FLU) with or without MF59 adjuvant. Data were as follows (see also Figure 51), where four mice were tested per group and virus titers are expressed as log_{10} $TCID_{50}$ per gram of tissue:

Immunogen	Virus replication in lungs of challenged mice		Virus replication in nasal turbinates of challenged mice	
	# infected/ # tested	Mean (± SE) virus titer	# infected/ # tested	Mean (± SE) virus titer
PBS	4/4	6.3 ± 0.3	3/4	2.8 ± 0.35
MF-59 alone	4/4	6.1 ± 0.13	3/4	3.0 ± 0.38
FLU vaccine (5 μg)	4/4	6.3 ± 0.07	3/4	2.9 ± 0.36
FLU vaccine (5 μg) + MF-59	4/4	6.0 ± 0.19	4/4	3.0 ± 0.11
BPL-SARS-CoV (5 μg)	¼	1.6 ± 0.13 *	0/4	Not detected **
BPL-SARS-CoV (5 μg) + MF-59	0/4	Not detected *	0/4	Not detected **

Two-tailed Student's t-test, compared to PBS-immunized mice, showed: * P<0.00001 or ** P=0.025

As shown, virus could not be detected in the BPL-SARS-CoV immunized mice. The lower limit of detection of infectious virus in a 10% w/v suspension of lung homogenate was 1.5 $log_{10}TCID_{50}$/gm, and in a 5% w/v suspension of nasal turbinates the limit was 1.8 $log_{10}TCID_{50}$/gm. Viral titers in the immunized mammals were thus below these threshold values.

Thus the inactivated SARS-CoV vaccine was very efficient at preventing virus infection, as only one of eight mice immunized with the vaccine, either with or without MF59 adjuvant, was infected. Similar protection was not observed in control groups of PBS diluent, MF59 adjuvant, or influenza virus vaccine with or without adjuvant.

Neutralization titers of sera taken from the animals in the challenge study were assessed at two weeks post-1st, one week post-2nd, and one week post-3rd immunization. Mice immunized with the vaccine with MF59 adjuvant had already developed a neutralization titer of 1:71 after the 2nd immunization, which increased to 1:588 after the 3rd immunization, whereas mice receiving the unadjuvanted vaccine did not have any neutralizing activity post-2nd and a neutralization titer of 1:64 post-3rd immunization. Sera from mice in each of the control groups did not show any neutralization activity. These data clearly demonstrate not only the ability of the inactivated SARS-CoV vaccine to induce protective levels of SARS neutralizing antibodies, but also a beneficial effect of formulating the vaccine with adjuvant for elevated neutralization titers.

EXAMPLE 6 - Preparation of OMV comprising SARS viral antigens

E.coli were transfected with a plasmid of interest (encoding a SARS viral antigen). Single colonies harbouring the plasmid of interest were grown overnight at 37°C in 20 ml of LB/Amp

(100 μg/ml) liquid culture. Bacteria were diluted 1:30 in 1.0 L of fresh medium and grown at either 30°C or 37°C until the OD_{550} reached 0.6-0.8. Expression of recombinant protein was induced with IPTG at a final concentration of 1.0 mM. After incubation for 3 hours, bacteria were harvested by centrifugation at 8 000 x *g* for 15 minutes at 4°C and resuspended in 20 ml of 20 mM Tris-HCl (pH 7.5) and complete protease inhibitors (Boehringer-Mannheim™). All subsequent procedures were performed at 4°C or on ice.

Cells were disrupted by sonication using a Branson Sonifier 450 and centrifuged at 5 000 x *g* for 20 min to sediment unbroken cells and inclusion bodies. The supernatant, containing membranes and cellular debris, was centrifuged at 50000g (Beckman Ti50, 29 000 rpm) for 75 min, washed with 20 mM Bis-tris propane (pH 6.5), 1.0 M NaCl, 10% (v/v) glycerol and sedimented again at 50000g for 75 minutes. The pellet was resuspended in 20mM Tris-HCl (pH 7.5), 2.0% (v/v) Sarkosyl, complete protease inhibitor (1.0 mM EDTA, final concentration) and incubated for 20 minutes to dissolve inner membrane. Cellular debris was pelleted by centrifugation at 5000g for 10 min and the supernatant centrifuged at 75000g for 75 minutes (Beckman Ti50, 33000 rpm). Outer membrane vesicles were washed with 20 mM Tris-HCl (pH 7.5) and centrifuged at 75 000 x g for 75 minutes or overnight. The OMV was finally resuspended in 500 μl of 20 mM Tris-HCl (pH 7.5), 10% v/v glycerol. Protein concentration was estimated by standard Bradford Assay (Bio-Rad), while protein concentration of inner membrane fraction was determined with the DC protein assay (Bio-Rad). Various fractions from the isolation procedure were assayed by SDS-PAGE.

EXAMPLE 7 - Immunogenicity, dose and route schedule for recombinant Spike protein in mice

The immunogenicity, route and dosing of the recombinant spike proteins of the invention in mice may be assessed using the below detailed protocol. Preferably, the administered antigen will elicit neutralizing antibody titers at least in the range of 1/100-1/1000. Increasing doses of antigen can be tested in the range from 5 to 20 μg of recombinant Spike antigen alone or mixed with an equal volume of MF59-citrate, administered SC or IM to anesthetized mice in 100 μl of inoculum. Groups of BALB/c mice, 6 per treatment are primed at day 0 and boosted at day 14 and 28.

Group	Treatment	Dose/Route	Sampling interval	Number of mice
1-3	Rec-Spike protein	20, 10, 5 μg/SC	7, 21, 35, 42 d	6 per dose level
4-6	Rec-Spike protein	20, 10, 5 μg/SC	7	6 per dose level
7-9	Rec-Spike protein	20, 10, 5 μg/IM	7, 21, 35, 42 d	6 per dose level
10-12	Rec-Spike protein	20, 10, 5 μg/IM	7	6 per dose level
13-15	Rec-Spike - MF59	20, 10, 5 μg/SC	7, 21, 35, 42 d	6 per dose level
16-18	Rec-Spike - MF59	20, 10, 5 μg/SC	7	6 per dose level
19-21	Rec-Spike - MF59	20, 10, 5 μg/IM	7, 21, 35, 42 d	6 per dose level
22-24	Rec-Spike - MF59	20, 10, 5 μg/IM	7	6 per dose level
25	MF59	NA/SC	7, 21, 35, 42 d	6 + 6 (sac d 7 and 42)
27	MF59	NA/IM	7, 21, 35, 42 d	6 + 6 (sac d 7 and 42)
29	Saline	NA/SC	7, 21, 35, 42 d	6 + 6 (sac d 7 and 42)
31	Saline	NA/IM	7, 21, 35,42 d	6 + 6 (sac d 7 and 42)

This protocol can also be used to assess the Th1/Th2 profile of the specific immune response elicited by the recombinant Spike protein. Neutralizing and Spike-specific antibody titers will be assessed at days 7, 21, and 35; IgG2a *vs* IgG1 isotype of the Spike-specific antibodies will be determined at days 21 and 35; *in vitro* proliferation of lymph node and splenic T cells against the recombinant Spike protein will be determined at days 7 and 42, respectively; IFN-γ and IL-4 production by splenic T cell against the recombinant Spike protein from SARS-CoV will be assessed at day 42. Peripheral blood will be collected at days 7, 21, 35; lymph nodes cells at day 7, and spleen cells at day 42. Neutralizing and Spike-specific antibody titers and isotypes will be determined by inhibition of SARS-CoV infection of Vero cells and by ELISA, respectively. Proliferation of lymph node and splenic cells will be determined by ^{3}[H]–Thymidine uptake. Frequencies of splenic IFN-γ and IL-4 producing T lymphocytes, will be determined by ELISPOT and FACS.

EXAMPLE 8 -Immunogenicity, dosing and route schedule for Spike proteins in rabbits

The immunogenicity, route and dosing of the recombinant spike proteins of the invention in rabbits may be assessed using the below detailed protocol. Increasing doses can be tested in the range from 5 to 40 μg of recombinant Spike antigen alone or mixed with an equal volume of MF59-citrate, administered SC or IM to anesthetized animals in 200μl of inoculum. Groups of New Zealand white female rabbits, 10 per treatment, will be immunized as shown in the table below. The animals will be primed at day 0 and boosted at days 14 and 28. Peripheral blood will be collected at days 7, 21, and 35. Neutralizing and Spike-specific antibody titers will be determined by inhibition of SARS-CoV infection of Vero cells and by ELISA, respectively.

Group	Treatment	Dose/Route	Sampling interval	Number of rabbits
1-4	Full-length Spike protein	40, 20, 10, 5μg/SC	7, 21, 35 d	10 per dose level
5-8	Full-length Spike protein	40, 20, 10, 5μg/IM	7, 21, 35 d	10 per dose level
9-12	Truncated Spike protein	40, 20, 10, 5μg/SC	7, 21, 35 d	10 per dose level
13-16	Truncated Spike protein	40, 20, 10, 5μg/IM	7, 21, 35 d	10 per dose level
17-20	Full-length Spike protein - MF59	40, 20, 10, 5μg/SC	7, 21, 35 d	10 per dose level
21-24	Full-length Spike protein - MF59	40, 20, 10, 5μg/IM	7, 21, 35 d	10 per dose level
25-28	Truncated Spike protein - MF59	40, 20, 10, 5μg/SC	7, 21, 35 d	10 per dose level
29-32	Truncated Spike protein - MF59	40, 20, 10, 5μg/IM	7, 21, 35 d	10 per dose level
33	MF59	NA/SC	7, 21, 35 d	10
34	MF59	NA/IM	7, 21, 35 d	10
35	Saline	NA/SC	7, 21, 35 d	10
36	Saline	NA/IM	7, 21, 35 d	10

EXAMPLE 9 - Immunogenicity and dose schedule for recombinant Spike in ferrets

The immunogenicity and dosing of the recombinant spike proteins of the invention in ferrets may be assessed using the below detailed protocol. Three groups of ferrets, 6 for treatment, will be immunized with recombinant SARS-CoV Spike protein from CHO cell lines, alone or mixed with an equal volume of MF59-citrate, administered SC to anesthetized animals in 200μl of inoculum. The recombinant Spike protein vaccine will be tested at the dose eliciting the highest neutralizing antibody titers in mice at day 35 after the second boost. The animals will be primed at day 0 and boosted at day 14 and 28. Peripheral blood will be collected at days 7, 21, and 35. Neutralizing and Spike-specific antibodies titers will be determined by inhibition of SARS-CoV infection of Vero cells and by ELISA, respectively.

Groups	Treatment	Dose/Route	Sampling interval	Number of ferrets
1 & 2	Rec-Spike protein	Y μg or 2Y μg /SC	7, 21, 35 d	6
3 & 4	Rec-Spike protein + MF59	Y μg or 2Y μg/SC	7, 21, 35 d	6
5	Saline	NA/SC	7, 21, 35 d	6

The 3 groups of ferrets, 6 animals per group, used for the immunogenicity studies above can then be used to assess efficacy of the recombinant Spike protein in protecting vaccinated animals from infection and/or disease. Anestethized animals will be challenged two weks after the last boost intratracheally with 10^6 median tissue culture infectious dose unit ($TCID_{50}$) of the SARS-CoV Utah strain. Infection by SARS-CoV will be assessed by taking nasal, faringeal and rectal swabs from animals for 20 days after challenge as described (12). The presence of SARS-CoV in sample materials will be assessed by RT-PCR and infection assay of Vero cells. Animals will be monitored for clinical signs of SARS disease by assessing sleeping time, temperature, respiratory symptoms, diarrhea, body weight and survival. Protection will be determined by the magnitude and duration of virus shedding and by duration and severity of disease symptoms and percentages of surviving animals.

EXAMPLE 10: Expression of Spike protein for vaccination

The SARS-CoV Spike glycoprotein was expressed in both full-length and truncated forms, using the nSh and nShΔTC pCMVIII constructs described above, both with hexahistidine tags. The vector constructs were evaluated for expression 48 hr after transfection into 293 cells and COS7 cells. The full-length Spike protein (nSh) was detected by western blot only in cell lysate, but not in culture media (Figure 52).

The majority of SARS-CoV full-length Spike protein was expressed in transiently-transfected COS7 cells as a high molecular glycoprotein which ran at 540 kDa in non-reducing gels (Figure 53). The gp540 is heat labile as indicated by the complete dissociation into monomeric forms (gp170 & gp180) by boiling, but it was resistant to DTT treatment. These data suggest that the recombinant Spike protein is noncovalently associated into a homotrimer (gp540). The presence of Spike protein in homotrimeric association also was confirmed in inactivated, purified SARS-CoV virion particles. Analysis of virion proteins by western blot under the same condition used for the characterization of recombinant Spike protein generated essentially identical results (Figure 54).

EXAMPLE 11: Spike protein processing

In order to characterize Spike protein processing, BHK-21 cells were infected with alphavirus replicon particles expressing the SARS-CoV full-length Spike. At 6 hoursr post-infection with an MOI of 5, infected cells were labeled for 1 hr with L-[^{35}S]methionine/cysteine and chased for up to 4 hours. The [^{35}S]-labeled spike protein was immunoprecipitated by anti-SARS rabbit serum and digested with Endo-H. Both digested and undigested proteins were analyzed by SDS-PAGE (4% polyacrylamide). As shown in Figure 55, the full-length spike protein is synthesized as an Endo-H sensitive high-mannose glycoprotein (gp170, an ER form) that undergoes modification to an Endo-H resistant glycoprotein with complex oligosaccharides (gp180, a Golgi form). The conversion of gp170 into the gp180 form takes place within 2 hours (Figure 56).

EXAMPLE 12: High-level protein expression

To develop a system for rapid expression of protein antigens, DNA transfection of 293 (human embryonic kidney) cells was used, to obtain milligram quantities of recombinant antigen. The most common method for culturing and transfecting 293 cells is in static or monolayer cultures. These procedures were modified by performing large-scale transfection of 293 cells in suspension and expanding the transfected cells in suspension culture for production of secreted or intracellular proteins. Several initial experiments were performed at the 100-milliliter scale cultures to determine optimum conditions, such as number of cells, type of

transfecting reagent (FuGENE 6, Lipitoid or RO-1538) and the ratio of DNA to transfection reagent. Based upon pilot experiments, FuGENE 6 was the best transfecting reagent.

The kinetics of gene expression was compared to other viral envelope glycoproteins, and the data suggest that stable protein expression peaks around 72 to 96 hours post-transfection, depending upon the gene of interest, and then significantly decreases thereafter. Thus, using the optimum conditions, the transfection process was scaled from 100 ml to 4 liters. The 4 liter culture can be used for rapidly producing 2-10 milligrams of protein antigens. To facilitate antigen purification and also maximize the yield and recovery of the purified protein, transfection conditions were optimized by using serum-free medium.

Bulk transfection procedure has been used for the expression of truncated and full-length Spike antigens. The kinetics of expression for truncated form of the spike protein is presented in Figure 56A. Expression of the truncated form of Spike protein peaked around 48 hrs and was stable until 72 hrs, therefore the cultures were harvested at 72 hrs post transfection.

Collected media were concentrated 20X and used for purification of truncated Spike protein by a very simple purification strategy where the truncated form of the spike was captured on GNA lectin followed by DEAE and ceramic hydroxyapatite column chromatography. The purified protein was analyzed on SDS-PAGE by silver stain (Figure 56B) and also by western blot (Figure 56C). Early efforts were able to purify the truncated form of the spike protein with >95% purity and approximately 50% recovery. The molecular mass of the truncated form of the Spike protein is approximately 170-180 kDa.

Full-length Spike protein was expressed in 293 cells using the bulk transfection strategy. The expression data suggest that, like the truncated form, expression peaked around 48 hrs post-transfection and remained stable until 72 hrs. However, contrary to the truncated form and as expected, full-length protein is not secreted, but rather is retained within the cells, as shown by the absence of any signal in western blots of cell culture supernatants. The full-length form of the protein was purified from Triton X-100 detergent-extracted cells. Full-length Spike protein was then captured on GNA lectin, followed by hydroxyapatite and SP chromatography. The calculated molecular mass of full-length spike protein is approximately 600 kDa, which is close to the theoretical mass for the trimer.

EXAMPLE 13: SARS virus seed cultures

A SARS-CoV reference seed virus propagated only in certified Vero cells will be used for the generation of the Master and Working Virus Seeds under GMP. A clinical specimen from the respiratory tract of a patient infected by the SARS-CoV is inoculated onto documented VERO cells, with certified culture media. Culture media containing the virus are harvested at 4 days post-infection and designated Passage 1 (P1). A second round of virus propagation is again

performed in certified VERO cells with certified media, by inoculation of 1 ml per T-75 flask of 100 times diluted P1 virus. Culture supernatant was harvested at 3 days post-infection and stored at -80°C as a P2 reference stock virus, without plaque purification.

Cell banks of Vero cells for further production of SARS-CoV are prepared from specific cell subsets that have not been used since the emergence of transmissible spongiform enephalopathies (*e.g.* since 1980). A research cell bank of these cells has been prepared using specified New Zealand-origin fetal bovine serum. From this research cell bank, a Master Cell Bank (MCB) is made under GMP conditions and using only specified and well-controlled media and supplements. The cell bank will is tested for absence of adventitious agents according to applicable US, EU, and international guidelines (see Points To Consider "Characterization of cell lines used to produce biologicals", FDA/CBER 7/1993; ICH Q5D Draft 6 "Cell substrates", Oct.23, 1996; CPMP/ICH/294/95 "Note for Guidance on Quality of Biotechnological Products: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products (Step 4, 16. July 97); WHO final draft "Requirements for use of animal cells as in vitro substrates for the production of biologicals" 7.3.1997). Tumorigenicity and identity testing is also required for this cell bank.

The reference virus is plaque-purified and expanded in certified Vero cells in the absence of FCS in order to generate Master and Working Seeds. Another option to help ensure purity and facilitate the assessment of safety of the Master Seed is to subject the SARS-CoV to pelleting and resuspension in PBS. The virus suspension is made up to 60% (w/w) sucrose with crystalline sucrose, transferred to a centrifuge tube and overlayed with 50, 40, 30, and 20% (w/w) sucrose solutions in PBS. The gradient is centrifuged for 72h and then fractionated. The virus-containing fraction is diluted and the virions re-pelleted by ultracentrifugation. RNA from the virus pellet is isolated and transfected into certified Vero cells whereby the "infectious" positive-strand RNA will lead to the production of infectious virus, which can be plaque-purified and expanded to generate alternative Master and Working Seeds from purified virus RNA.

Viral seeds are tested for the absence of adventitious agents (see e.g. 21 CFR Revised as of April 1, 1994, § 630.35 Test for safety) and for identity, using a highly-specific neutralizing antiserum prepared from an independent source. Safety testing of viral seeds for vaccine purposes is done routinely by service laboratories. Broad-spectrum PCR testing can be used as an addition and/or alternative for testing.

EXAMPLE 14: Scale-up of virus production and inactivation

A protocol for the production, inactivation, and purification of inactivated SARS-CoV with sufficient structural integrity to elicit protective neutralizing antibody responses in animal models involves: Vero cells are infected with virus at an M.O.I. of 0.01 in the absence of FCS

and antibiotics; culture medium is collected, cleared by centrifugation, and inactivated with BPL, followed by confirmatory testing for complete inactivation; the inactivated material is filtered, subjected to MCS-column purification, and further purified by sucrose gradient centrifugation.

Several modifications and improvements can be developed when adapting this basic protocol to a larger scale for commercial use. Firstly, the cell culture and infection process can be adapted to roller bottles, as an intermediate step to allow rapid production for preliminary trials within existing BSL 3+ facilities. Full commercial production will typically use a fermentation process in a closed system, but a roller bottle system can be achieved more rapidly. The roller bottles do offer a true suspension culture system for Vero cells, which gives various technical and safety advantages over microcarrier cultures. Suspension cultures can be grown to any desired fermentation scale without interfering with the closed system between cell passages, as no trypsinization is required.

To scale up the infection process in roller bottles to 30-50 liters per batch, the optimum M.O.I. and harvesting periods for selected media and culture conditions should first be determined. For the larger scale, methods for harvesting and handling larger volumes of highly infectious material safely should be used, and so cell separation via centrifugation should be replaced by a method such as filtration through single-use filter cartridges.

The MCS-chromatography and the gradient purification steps described above can readily be scaled to a batch volume of up to 50 liters. For larger volumes, however, and for increased purity, ultrafiltration and sterile filtration steps will be used. Nuclease treatment to remove host cell DNA will also be included.

EXAMPLE 15: Large scale analytical methods

Analytical methods for the SARS coronavirus include virus titration methods, immunological and physico-chemical methods to quantitate and characterize the purified antigen (ELISA, PAGE, western blots using specific antisera against purified whole virus, *etc.*). Other analytical tests include: fast yield testing via asymmetric field flow separation and laser particle detection and counting; Western blot using specific antisera against individual viral proteins; and tests for residual host cell DNA.

Residual DNA testing is generally done by hybridization *e.g.* using a limit test. Such testing is performed according to methods already established and validated for other cell lines. As an alternative, the Threshold™ method may be used.

For producing specific antibodies, recombinant protein expression of all the ORFs from the structural and non-structural gene regions of the SARS-CoV is used. The ORFs can be cloned and expressed in *E.coli* and, if necessary, also in eukaryotic vectors such as baculovirus. This can provide sufficient amounts of purified soluble protein to immunize mice and rabbits to produce

polyclonal and monoclonal antibodies against SARS proteins and to set up specific ELISA assays. Different expression vectors can be tested to maximize the yield of recombinant protein in a soluble form *e.g.* different vectors, one containing sequences coding for six N-terminal histidine residues and another containing a Glutathione-S-transferase protein fused to the C-terminus of the SARS protein. The recombinant proteins can be purified by single step column chromatography on either Nickel chelating Sepharose or Glutathione-Sepharose 4B resin. These procedures are very rapid and generally produce protein of 60-90% purity, which is suitable for raising specific antisera (Pizza *et al.* (2000) *Science* 287:1816-20). Five mice and two rabbits for each recombinant protein can be immunized SC with 20 and 50 μg recombinant protein, respectively, given in IFA as adjuvant, at day 0, 14 and 28. Sera are collected at day 7, 21 and 35 to assess specific titers before euthanasia of the animals for collection of blood and removal of spleens.

For the detection of impurities (*e.g.* Vero cell derived proteins) in the vaccine preparation, rabbit serum reactive against Vero-derived proteins can be used. Such antisera are obtained by immunizing rabbits with at least 10μg of Vero cell lysate with CFA/IFA. The sera can be verified for reactivity against Vero-derived proteins in western blots. For more specific antisera against specific relevant cell-derived proteins that tend to be co-purified with the virus, mock-infected cell culture harvest that have undergone the purification process can be prepared and used for immunizing rabbits.

Methods to determine neutralization titers of sera from immunized animals and humans can be developed, without the constraints of using infectious SARS-CoV in a BSL-3+ laboratory. One such strategy will be to use recombinant antigens, particularly Spike protein or Spike-derived epitopes, and to develop ELISA assays for measuring antibodies against the target protein. Suitable epitopes allow a correlation to be established between the ELISA values and virus neutralization assay values. This approach provides a faster and more efficient (higher-throughput) comparison of specific and protective antibody titers. This ELISA test is also the ideal tool to monitor specific antibodies in safety trials, where several hundred animal sera must be tested.

Another strategy is to combine structural elements from both the pathogenic SARS-CoV and the non-pathogenic coronavirus mouse hepatitis virus (MHV) to construct chimeric virus-like particles (VLPs) that can be labeled. The assay is based on fusion between octadecyl rhodamine (R18)-labeled VLPs and cells (Hoekstra *et al.* (1984) *Biochemistry* 23:5675-81). The method relies on the relief of fluorescence self-quenching of R18 incorporated into VLPs upon fusion with cellular membranes. Coronavirus VLPs have been shown to mimic native virions with respect to their appearance in the electron microscope (EM) and their biological activities. As they do not contain viral RNA, however, then they cannot cause a productive infection

(Vennema *et al.* (1996) *EMBO J* 15:2020-2028). The VLP system can be used for the mouse hepatitis virus (MHV) strain A59 (MHV-A59))Godeke *et al.* (2000) *J Virol* 74:1566-15) containing a chimeric S protein. The protein chimera, consisting of the ectodomain of the SARS-CoV and the transmembrane and endodomain (64 C-terminal amino acid residues) from the MHV spike protein, can be co-expressed with the MHV M (membrane) and E (envelope) protein in OST-7 cells)Godeke *et al.*). VLPs secreted in the supernatant are harvested, purified and labeled with octadecyl rhodamine (R18) (Hoekstra *et al*). A constant amount of VLPs is incubated with a serial dilution of sera at 37°C for 1 hour in a 96-well plate. Subsequently, cells expressing the receptor for the SARS-CoV, the angiotensin-converting enzyme 2 (ACE2) (Li *et al.* (2003) *Nature* 426:450-54) is be added and the extent of fusion can be measured with a fluorescence spectrophotometer.

A final strategy to monitor the ability of sera to inhibit cell-cell fusion interactions between cells expressing the SARS-CoV S protein and a human cell line expressing the angiotensin-converting enzyme 2 (ACE2), a functional receptor for SARS-CoV (Li *et al.*). This reporter gene-based assay uses the fluorescent shift (green to blue) of the fluorogenic substrate CCF2/AM (AM=acetoxymethyl) upon cleavage by β-lactamase (Bla) as read-out for cell-cell fusion (Zlokarnik *et al.* (1998) *Science* 279:84-88). For this assay, a BHK-derived cell line, stably expressing Bla and the SARS-CoV S protein is generated. In addition, a human cell line expressing ACE2 on its surface is used. BHK cells, expressing the S protein on their surface and Bla in their cytosol are incubated with serial dilutions of the sera to be tested for 1h at 37°C. The cell line expressing the ACE2 is loaded with 1μM CCF2/AM for 1 h at 22°C, washed twice with PBS, and co-cultivated with the BHK cells. In case of cell-cell fusion, Bla cleaves the substrate, resulting in a green blue shift with excitation at 409 nm. Inhibition of fusion by sera thus provides a detectable change.

EXAMPLE 16: Stabilisation of inactivated SARS-CoV

Although the purified inactivated SARS-CoV vaccine is capable of inducing potent neutralizing antibody responses in animals, it is relatively instable and can benefit from formulation to increase stability for an acceptable period of time. Suitable formulation changes include the use of various buffer systems, pH ranges, stabilizing excipients (*e.g.* sugars and sugar alcohols, amino acids, *etc.*) *etc.*. Stability testing can be conducted in real-time at normal storage temperatures, or can be conducted in an accelerated manner by using elevated temperatures. Vaccine stability can thus be increased to approximately one year or longer. Lyophilized vaccine formulation can also be used to extend shelf-life, possibly with further additives for stability during lyophilisation.

EXAMPLE 17: Dose and schedule optimization for inactivated virus

Animal models of SARS-CoV infection have been reported, including mice, ferrets and macaques. As mentioned in example 4 above, mice immunized with the BPL-SARS-CoV vaccine achieve neutralizing antibody titers in the range of 1:100 – 1:1000, similar to levels found in convalescent patients, and are 100% protected from infection with a challenge virus. While the mouse challenge model is limited only to infection but not disease, ferrets and macaques are useful models of the human SARS disease. Two to four days after inoculation with SARS-CoV, both ferrets and macaques have been found to shed infectious SARS-CoV particles from the throat, nose and pharynx, as demonstrated by RT-PCR and/or virus isolation on Vero cells. At approximately the same time, the infected animals became lethargic, show respiratory distress and eventually die. Histologically, SARS-CoV infection in these animals associates with pulmonary lesions of different severity, similar to those found in biopsied lung tissue and autopsy material from SARS patients. With the availability of these models, preclinical studies with vaccines can be performed initially in mice for immunogenicity readouts, while efficacy of optimal doses and schedules can be assessed in the ferret and macaque models.

Initial studies in mice are used to determine the optimal dose and schedule required to elicit the highest levels of neutralizing antibody, with titers at least in the range of 1/100 – 1/1000. In parallel to the assessment of neutralizing activity, other features of the humoral immune response and cellular immune responses can be investigated. In particular sera from immunized mice can be assessed for the isotype (IgG1 *vs.* IgG2a) of the Spike-specific antibody response. Also, the frequencies of splenic CD4+ T cells producing IFN-γ and IL-4 in response to BPL-SARS-CoV particles will be assessed by ELISPOT and ELISA. These experiments can provide insight into the quality of the T cell response helping the priming of a protective antibody response.

Increasing vaccine doses can be tested (*e.g.* from 5 to 20 μg of BPL-SARS-CoV alone or mixed with an equal volume of MF59-citrate), administered SC to anesthetized mice in 100μl of inoculum. Groups of BALB/c mice, 10 per treatment, are immunized, with priming at day 0 and boosting at days 14 and 28. Secondary endpoints compare the kinetics of neutralizing *vs.* Spike-specific antibody titers and assess the Th1/Th2 profile of the specific immune response, and so neutralizing and Spike-specific antibody titers are assessed at days 7, 21, 35, and at 2, 3, 4, and 5 months after priming. The IgG2a and IgG1 titers of Spike-specific antibodies are determined at days 21, 35, and at 2, 3, 4, and 5 months after priming. Proliferation and IFN-γ and IL-4 production by splenic T cells against recombinant Spike protein from SARS-CoV are assessed at day 42, and at the end of the 5th month. Peripheral blood is collected at days 7, 21, 35, and at 2, 3, 4, and 5 months after priming. Spleen cells will be obtained at day 42 and at the end of the 5th month. Neutralizing and Spike-specific antibody titers and isotypes are determined by inhibition

of infection of Vero cells and by ELISA, respectively. Proliferation of splenic cells is determined by ^{3}[H]-thymidine uptake. Frequencies of splenic IFN-γ and IL-4 producing $CD4^{+}$ T lymphocytes is determined by ELISPOT and FACS analysis.

Based on mouse results, the BPL-SARS-CoV vaccine can be tested in ferrets for the induction of protective neutralizing antibody titers. Ferrets are immunized according to a similar schedule as the mice and at the dose that elicits the highest neutralizing antibody titers in mice at day 35 after the second boost. Three groups of ferrets, 6 per treatment, are immunized with BPL-SARS-CoV, alone or mixed with an equal volume of MF59-citrate, administered SC to anesthetized animals in 200μl of inoculum. The animals are primed at day 0 and boosted at days 14 and 28. Peripheral blood is collected at days 7, 21, and 35. Neutralizing and Spike-specific antibodies titers are determined by inhibition of SARS-CoV infection of Vero cells and by ELISA, respectively. Each group of ferrets is used to assess efficacy of the BPL-SARS-CoV in protecting vaccinated animals from infection and/or disease. Anesthetized animals are challenged intratracheally, two weeks after the last boost, with 10^{6} median tissue culture infectious dose units ($TCID_{50}$) of the SARS-CoV CDC strain. Infection by SARS-CoV can be assessed by taking nasal, pharyngeal and rectal swabs from animals for 20 days after challenge (Martina *et al. supra*). The presence of SARS-CoV in sample materials can be assessed by RT-PCR and infection assay of Vero cells. Animals can be monitored for clinical signs of SARS disease by assessing sleeping time, temperature, respiratory symptoms, diarrhea, body weight and survival. Protection can be determined by the magnitude and duration of virus shedding, by duration and severity of disease symptoms, and by percentage of surviving animals. The formulation eliciting the highest neutralizing antibody titers at day 35 can then be tested against a two-fold higher dose of BPL-SARS-CoV given in the same formulation in the same regimen.

Additional studies can evaluate immunogenicity and efficacy of the candidate vaccine in non-human primates. Three groups of adult cynomolgus macaques, 4 per treatment, are immunized with BPL-SARS-CoV, alone or mixed with an equal volume of MF59-citrate, administered SC to anesthetized animals in 500μl of inoculum. The BPL-SARS-CoV vaccine can be tested at the dose eliciting the highest neutralizing antibody titers in ferrets at day 35 after the second boost. The animals are primed at day 0 and boosted at 3 and 6 weeks. Peripheral blood is collected at weeks 1, 4, and 7. A secondary endpoint is to assess the Th1/Th2 profile of the specific immune response. Neutralizing and Spike-specific antibody titers and frequencies of peripheral blood CD4+ T cells producing IFN-γ and IL-4 in response to the recombinant SARS-CoV Spike protein is thus assessed at weeks 1, 4, and 7. Neutralizing and Spike-specific antibody titers can be determined by inhibition of SARS-CoV infection of Vero cells and by ELISA, respectively. Intracellular cytokine staining and FACS analysis will be used to quantify IFN-γ- and IL-4-producing $CD4^{+}$ T cells. The macaques can also be used to assess efficacy of

the BPL-SARS-CoV in protecting vaccinated animals from infection and/or disease. Anesthetized macaques can be challenged two weeks after the last boost with 10^6 median tissue culture infectious dose unit ($TCID_{50}$) of the SARS-CoV CDC strain in a 5 ml volume. A few drops of the virus can also be administered on each of the conjunctiva, 0.5 ml in the nose and the remainder in the trachea. Infection by SARS-CoV can be assessed by taking nasal, pharyngeal, and rectal swabs, and feces from animals for 20 days after challenge (Fouchier *et al.* (20030 *Nature* 423:240). The presence of SARS-CoV in sample materials can be assessed by RT-PCR and infection assay of Vero cells. Animals can also be monitored for clinical signs of SARS disease by assessing sleeping time, temperature, respiratory symptoms, diarrhea, body weight and survival. Protection can be determined by the magnitude and duration of virus shedding, by duration and severity of disease symptoms, and by percentage of surviving animals.

Mice

Group	Treatment	Dose/Route	Sampling interval	Number of mice
1-3	BPL-SARS-CoV	20, 10, 5 μg/SC	7, 21, 35 d; 2, 3, 4, 5 m;	10 per dose level
4-6	BPL-SARS-CoV	20, 10, 5 μg/SC	42 d	10 per dose level
7-9	BPL-SARS-CoV MF59	20, 10, 5 μg/SC	7, 21, 35 d; 2, 3, 4, 5 m;	10 per dose level
10-12	BPL-SARS-CoV MF59	20, 10, 5 μg/SC	42 d	10 per dose level
13	MF59	NA/SC	7, 21, 35 d; 2, 3, 4, 5 m;	10 + 10 (sacrificed at 42 d and end 5 m)
14	Saline	NA/SC	7, 21, 35 d; 2, 3, 4, 5 m;	10 + 10 (sacrificed at 42 d and end 5 m)

Ferrets

Group	Treatment	Route	Sampling interval	No. of ferrets
1	BPL-SARS-CoV	SC	7, 21, 35 d	6
2	BPL-SARS-CoV-MF59	SC	7, 21, 35 d	6
3	Saline	SC	7, 21, 35 d	6

Macaques

Group	Treatment	Route	Sampling interval	No. of macaques
1	BPL-SARS-CoV	SC	1,4, 7 w	4
2	BPL-SARS-CoV – MF59	SC	1,4, 7 w	4
3	Saline	SC	1,4, 7 w	4

EXAMPLE 18: Human T cell responses

As a prelude to initiation of clinical studies in humans, the reactivity of peripheral blood T lymphocytes from healthy donors with different HLA haplotypes can be assessed using the *in vitro* priming technique (Abrignani *et al.* (1990) *Proc Natl Acad Sci U S A* 87:6136-40). The aim of this study is to have a first indication of the immune-dominant T cell epitopes in SARS-CoV

proteins. Briefly PBMCs from 20 healthy donors with different HLA haplotypes will be cultured in medium containing 5% autologous serum, in the presence of different concentration of SARS-BPL-CoV particles in the range from 0.5 to 20 μg/ml. The expression of activation markers will be assessed after 24 and 48 hours. Frequencies of IFN-γ- and IL-4- producing T lymphocytes will be assessed after 12h and after 15 days in culture, in the presence of 100 U/ml recombinant human IL-2. Activated and cytokines producing CD4 T lymphocytes will be sorted and eventually cloned as single cells using FACS technologies. The CD4+ T cell repertoire from human subjects with different HLA will be assessed by proliferation assays of the CD4+ T cell lines and clones against autologous EBV-transformed cell lines loaded with 15-mer overlapping peptides from the most relevant structural and non structural protein of the SARS-CoV.

When moving to actual human trials, safety and immune responses will be evaluated in healthy adults following intramuscular immunization with escalating doses of the BPL-inactivated SARS-CoV vaccine, with MF59 adjuvant being included or omitted depending on preclinical data. Three/four immunizations will be given at 0, 1, 6 months in the first cohort, and at 0, 1, 2, 6 months and 0, 2, 6 weeks in the second and third cohorts respectively. The trial will be observer blind and placebo controlled. Subjects will be randomized into each dose level. Immune response parameters to be measured will include serum neutralizing antibodies, ELISA antibodies and peripheral blood IFN-gamma-producing CD4+ T cells by intracellular cytokine staining.

Group	Antigen dose (μg)	Administration schedule	No. treated subjects	No. subjects with placebo	Sampling interval
A1	10	0,1,6 months	18	6	0, 1, 2, 6, 7 mos
A2	20	0,1,6 months	18	6	0, 1, 2, 6, 7 mos
B1	10	0,1,2,6 months	18	12	0, 1, 2, 6, 7 mos
B2	20	0,1,2,6 months	18	12	0, 1, 2, 6, 7 mos
C1	10	0,2,6 weeks	18	12	0, 2, 6, 10, 30 wks
C2	20	0,2,6 weeks	18	12	0, 2, 6, 10, 30 wks

EXAMPLE 19: Selection of CHO cell lines for Spike protein expression

Methods for the derivation of Chinese Hamster Ovary (CHO) cell lines that stably express viral envelope glycoproteins that are conformationally intact, appropriately glycosylated and efficiently bind neutralizing antibodies are well established for HIV and HCV (Srivastava *et al.* (2002) *J Virol* 76:2835-47; Srivastava *et al.* (2003) *J Virol* 77:11244-259; Heile *et al.* (2000) *J Virol* 74:6885-92). The same techniques can be applied to SARS-CoV, to generate two different stable CHOK-1 cell lines producing either full-length or truncated SARS Spike proteins. The Spike proteins can be expressed using the constructs described herein, but without the hexa-His tags. These proteins can compared for their ability to produce neutralizing antibodies in immunized animals as well as for their expression levels in CHOK-1 cells.

A pCMV3 vector expressing Spike can be used for the derivation of stable CHOK-1 cell lines, containing the CMV enhancer/promoter, ampicillin resistance, and a fused DHFR and attenuated neomycin gene for selection purposes. Stable cell lines can produced using the neomycin selection system in CHOK-1 cells. Clones can be sequenced to verify the integrity of the insert, and transient transfections can be performed using Trans-LT1 polyamine transfection reagent (PanVera Corp., Madison, WI) to assess the expression level and also the integrity of the expressed protein by ELISA and western blot analysis.

Initial CHO cells will be selected to be free from TSE/BSE contaminants and risks according to relevant regulatory standards. To construct cell lines, procedures involve transfection, primary screening with selective medium, followed by subcloning to assure purity of cell lines. Cell supernatants can be assayed using an antigen capture ELISA to quantify expression levels at all stages of selection and amplification. For full-length Spike expression, methanol fixed cells can be screened for internal expression by immunofluorescent staining using a rabbit anti-SARS antibody. Successive measurements at the T75-flask stage of expansion canbe employed to assure stability of expression levels. The molecular mass and integrity of the expressed proteins can be checked by PAGE both under native and reducing and denaturing conditions, followed by immunoprobing.

The pCMV3 vectors expressing SARS-CoV Spike proteins in either full-length or truncated forms can be introduced into CHOK-1 cells using the Trans-LT-1 reagent and non-selective media. 24-48 hours post-transfection, depending on cell density, cells are split at a 1:5 ratio and the medium can be changed to selective media containing neomycin at 500μg/ml. Any bovine serum used in these procedures will be from TSE-free sources that meet regulatory standards. Ten to fourteen days later, individual colonies can be picked and transferred to 96 well plates and cultured in complete non-selective medium. When approximately 80% of the wells are confluent, 24 hour supernatants can be screened by Spike capture ELISA. For initial expression of full length Spike protein, cells can be fixed with methanol and screened by immunofluorescent staining using a rabbit anti-SARS antibody. After low-expressing cell lines have been eliminated and there are fewer than 20-30 cell lines, capture ELISA and western blots can then be used to determine the expression level after cell lysis. A portion of each cell line can be pelleted, weighed and lysed in 1% Triton lysis buffer for determination of expression levels. Three to four clones producing the highest levels of spike protein in correct structure and conformation can be expanded to three-liter bioreactors and adapted to low serum suspension culture conditions for scale-up.

The antigen capture ELISA assay for the SARS spike protein can be performed using 96 well flat-bottom plates coated with 250ng per well of purified immunoglobulin obtained from rabbit sera that were immunized with inactivated SARS virus. Supernatant or lysate samples are

added and incubated for 2 hours at 37°C. Bound antigen is reacted against pooled $SARS^{+ve}$ serum or high affinity monoclonal antibody either human or mouse against SARS spike protein and detected using appropriate species-specific peroxidase-conjugated second antibody. The plates are developed using TMB substrate (Pierce, Rockford, IL), read at a wavelength of 450nm, and the concentration of protein per ml sample is derived from a standard curve (OD *vs.* protein concentration) based on serial dilutions of a known concentration of recombinant spike protein.

The immunoprobing analysis will also be performed following the standard methods described by Srivastava *et al.* (2002) *supra*. Briefly, 10-20μl of the sample is analyzed on 4-20% SDS PAGE under non-reducing/denaturing conditions with mild heating. The proteins are then transferred onto nitrocellulose membranes and reacted against polyclonal anti-Spike rabbit serum, followed by anti-rabbit Ig conjugated to Alexa 688 (Molecular Probes, Oregon). The blots are scanned using an infrared imaging system.

The highest expressing candidate cell lines will be screened for Spike protein expression and stability in small-scale (3 liter) perfusion bioreactors. The candidate clones will be further evaluated for level of expression as well as integrity of expressed protein, and subsequently tested for expression stability in the absence of selection. The selected clones also will be tested for maintenance of the DNA sequence integrity of the integrated SARS spike protein gene. To quickly monitor the expression levels in small flasks and in the three liter evaluation cultures, a lectin-based process (Gluvanthus Nivalis lectin) has been developed to isolate SARS spike protein to a degree of purity that allows semi-quantitation and characterization of the protein in CHO supernatant. Full-length Spike protein will be obtained from Triton X-100 detergent extracted cells and then captured on GNA lectin, followed by hydroxyapatite and SP chromatograph. Eluted protein is then characterized by: (1) polyacrylamide gel electrophoresis (PAGE) and Coomassie staining, (2) immunoprobing with anti-SARS rabbit sera, (3) structural characterization using size exclusion chromatography (SEC), as well as mass spec analysis using MALDI-TOF.

Productivity from the CHO cell line expressing SARS spike protein should be at least 2 mg/L and for full-length Spike protein will be 3mg/100gm of cells, at steady-state cell density. Yield from one 45 day, 2.5-liter bioreactor will be ~1000 mg crude protein.

EXAMPLE 20: Purification of spike protein for human vaccines

To purify SARS spike protein for the purpose of producing GMP grade material for human use, the following basic process is used, with all steps being performed at 2-8°C: the starting material, concentrated CHO cell culture supernatant (20-30X) is thawed and filtered through a 0.45μm membrane; this material is heavily contaminated proteins from culture, as well as DNA;

the first purification step is affinity chromatography using Gluvanthus Nivalis (GNA), a lectin that preferentially recognizes terminal mannose containing carbohydrates; glycosylated proteins, including SARS spike protein are captured and non-glycosylated proteins, as well as DNA, do not bind to this column; the GNA column is followed by two chromatographic steps operated in the flow through mode; the anion exchanger, DEAE, and ceramic hydroxyapatite (cHAP); DEAE binds some contaminating supernatant proteins and DNA, whereas cHAP binds any contaminating serum proteins; full-length Spike protein is purified from the cell pellet; the cells are lysed with Triton X-100 and full-length Spike protein is then captured on GNA lectin, followed by hydroxyapatite and SP chromatography.

The purified SARS spike can be further treated to remove adventitious viruses: viral inactivation at pH 3.5 for 1 hour; the sample is then concentrated and diafiltered into a buffer at pH 4 and finally captured the purified protein using SP resin; the spike protein binds to this resin and many viruses flow through.

The spike protein is eluted, concentrated and diafiltered into formulation buffer. This formulated bulk product is then filtered through a DV50 viral removal membrane followed by filtration through a 0.2 μm membrane. The formulated bulk is filled into suitable containers *e.g.* into 3.0 ml vials, in a class 100 laminar flow hood.

In process testing at each step of the purification includes protein concentration, endotoxin (LAL), bioburden, and recovery.

Prior to human administration, a test for potency will evaluate the specific ability of the vaccine in an *in vitro* or *in vivo* test to effect a given response. The *in vivo* immunogenicity will be determined by dosing groups of 10 mice with various doses of the protein antigen. Sera will be analyzed for the presence of IgG antibodies using an ELISA. The criterion for passing will be based upon the number of vaccine treated animals that are seropositive compared to a reference standard. Other tests include General Safety, sterility, purity, identity of the vaccine (using an ELISA specific for Spike protein), and quantity & protein concentration (UV spectrophotometric absorbance procedure based on the molar absorbance of the aromatic amino acids).

Stability testing will be performed on the bulk drug substance and on the final container product. Bulk product will be evaluated at temperatures of –60°C (recommended storage condition), 25 ±2 °C and 40 ±2 °C protected from light, at time points of 0, 3, 6, 9, 12 months. Final container product will be tested at temperatures of –60 °C, and inverted at 5 ±3 °C, 25 ±2 °C, and 40 ±2 °C at time points of 0, 3, 6, 9, 12 months. Stability-indicating assays may include appearance, pH, protein content, SDS-PAGE, size exclusion HPLC, and container/closure integrity, performed on single samples of bulk and triplicate vials of final container material.

The protein purified in this way can be evaluated in mice, rabbits and ferrets as described in, and based on the results of, examples 4, 5, 8 and 9 above.

Initial experiments will be performed in mice to determine optimal dose and schedule of the GMP Spike protein required to elicit the highest levels of neutralizing antibody, with titers at least in the range of 1/100 – 1/1000. Spike protein will be tested in the range from 5 to 40 μg, alone or mixed with an equal volume of MF59-citrate, to anesthetized mice in 100μl of inoculum. Groups of BALB/c mice, 10 per treatment, will be immunized. The animals will be primed at day 0 and boosted at days 14 and 28. Secondary endpoints will be to compare the kinetics of neutralizing *vs.* Spike-specific antibody titers and to assess the Th1/Th2 profile of the specific immune response. Neutralizing and Spike-specific antibody titers will be assessed at days 7, 21, and 35 and at 2, 3, 4, and 5 months after priming; the IgG2a and IgG1 titers of Spike-specific antibodies will be determined at days 21 and 35, and at 2, 3, 4, and 5 months after priming; proliferation and IFN-γ and IL-4 production by splenic T cell against the recombinant Spike protein from SARS-CoV will be assessed at day 42 and at the end of the 5th month. Peripheral blood will be collected at days 7, 21, and 35 and at 2, 3, 4, and 5 months after priming; spleen cells at day 42 and at the end of the 5th month. Neutralizing and Spike-specific antibody titers and isotypes will be determined by inhibition of SARS-CoV infection of Vero cells and by ELISA, respectively. Proliferation of splenic cells will be determined by ^{3}[H]–thymidine uptake. Frequencies of splenic IFN-γ and IL-4 producing CD4+ T lymphocytes, will be determined by ELISPOT and FACS analysis.

Next, the optimal dosing and schedule for recombinant Spike vaccine will be determined in ferrets. Based on the mouse results, the Spike vaccine eliciting the highest antibody neutralizing titers will be tested against a two-fold higher dose of recombinant Spike protein given in the same formulation. Three groups of ferrets, 6 per treatment, will be immunized SC under anesthesia with 200μl of inoculum. The animals will be primed at day 0 and boosted at days 14 and 28. Peripheral blood will be collected at days 7, 21, and 35. Neutralizing and Spike-specific antibodies titers will be determined by inhibition of SARS-CoV infection of Vero cells and by ELISA, respectively. Similar to the previous ferret studies, each group of animals will be used to assess efficacy of the vaccine in protecting immunized animals from infection and/or disease.

Immunogenicity and efficacy of the candidate vaccine also will be evaluated in nonhuman primates. Three groups of adult cynomolgus macaques, 4 per treatment, will be immunized with recombinant SARS-CoV Spike protein, alone or mixed with an equal volume of MF59-citrate, administered SC to anesthetized animals in 500 μl of inoculum. The Spike protein vaccine will be tested at the dose eliciting the highest neutralizing antibody titers in ferrets at day 35. The animals will be primed at day 0 and boosted at 3 and 6 weeks. Peripheral blood will be collected at weeks 1, 4, and 7. A secondary endpoint will be to assess the Th1/Th2 profile of the specific immune response, as described above (neutralizing and Spike-specific antibody titers,

frequencies of peripheral blood CD4+ T cells producing IFN-γ and IL-4 in response to the recombinant Spike protein, assessed at at weeks 1, 4, and 7).

Finally, human phase I, placebo-controlled, dose-escalation, safety/ immunogenicity trials will be performed for the IM recombinant SARS vaccine with MF59 adjuvant. The trial will evaluate safety and immune responses in healthy adults following immunization with escalating doses of SARS recombinant vaccine with MF59 adjuvant, administered intramuscularly. Three/four immunizations will be given at 0, 1, 6 months. The trial will be observer blind and placebo controlled. Subjects will be randomized into each dose level. Immune response parameters to be measured include serum neutralizing antibodies, ELISA antibodies and peripheral blood IFN-γ-producing CD4+ T cells by intracellular cytokine staining:

Group	Vaccine Antigen dose (μg)	Administration schedule	No. of treated subjects	No. of subjects with placebo (MF59)	Sampling interval
A1	50	0,1,6 months	18	6	0, 1, 2, 6, 7 months
A2	100	0,1,6 months	18	6	0, 1, 2, 6, 7 months

EXAMPLE 21: Comparison of inactivated virus and purified Spike protein

Immunogenicity and efficacy of the inactivated virus vaccine and the purified Spike protein can be compared in non-human primates. Three groups of adult cynomolgus macaques, 4 for treatment, will be immunized with recombinant SARS-CoV Spike protein from CHO cell lines or with BPL-SARS-COV, given in the dose and formulation eliciting the highest neutralizing antibody titers in previous immunogenicity challenge experiments, administered SC to anesthetized animals in 500μl of inoculum. The animals will be primed at day 0 and boosted at 3 and 6 weeks. Peripheral blood will be collected at weeks 1, 4, 7. A secondary endpoint will be to assess the Th1/Th2 profile of the specific immune response, as described above.

Group	Treatment	Dose/Route	Sampling interval	No. of macaques
1	Rec-Spike protein + or - MF59	Y μg /SC	1,4, 7 w	4
2	BPL-SARS-CoV + or - MF59	Y μg/SC	1,4, 7 w	4
3	Saline	NA/SC	1,4, 7 w	4

EXAMPLE 22: Expression in yeast

Yeast is a useful and inexpensive eukaryotic expression system. Yeast-expressed proteins are used in recombinant hepatitis B virus vaccines, and recombinant SARS antigens may also be expressed in yeast for vaccine purposes. Yeast-expression is also convenient for the production of antigens for preparing monoclonal and polyclonal antitobodies, or for use in serological assays.

The nucleocapsid protein (N) and two different versions of the spike glycoprotein (S) from SARS coronavirus FRA strain (AY310120) were cloned for expression in *S.cerevisiae:*

SARS N: aa 1 – 422 (coordinates 28120-29388 of AY310120 strain) – Fig.65

SARS spike: aa 14 – 1195 (transmembrane domain and cytoplasmic tail deleted) – Fig.66

SARS spike: aa 14 – 662 (S1 domain)

To make the S1 construct, a XhoI-NotI fragment of approximately 3733bp encoding the full-length spike glycoprotein was the starting point. PCR was used to amplify the full-length gene in two pieces: XbaI-BlnI of 2440bp and BlnI-SalI of 1306bp. These fragments were subcloned into commercial vectors (Novagen): pT7Blue2 XbaI-BlnI (5' end of spike glycoprotein) and pT7Blue2 BlnI-SalI (3' end of spike glycoprotein; Figure 58), respectively. The following primers were used in the subsequent PCR reactions: Spk-1 (5') SEQ ID NO: 9785; Spk-2 (5') SEQ ID NO: 9786; Spk-3 (5') SEQ ID NO: 9787; Spk-4 (5') SEQ ID NO: 9788.

E. coli HB101 competent cells were transformed with the PCR ligation product and plated on Luria agar plates, containing 100μg/ml ampicillin. The desired clones were identified using miniscreen DNA analysis. After sequence verification and plasmid amplification of the desired subclones, it was desirable to eliminate the internal SalI site present in the XbaI-BlnI portion of the spike sequence in order to facilitate future cloning into the yeast expression vector (BamHI-SalI). Therefore, we prepared a CelII-MfeI vector from the pT7Blue2 XbaI-BlnI (5' end Spike) subclone to eliminated a 143bp sequence containing the SalI site. Kinased oligos DS1-6 (SEQ ID NOS: 9789-9794) were then ligated into the CelII-MfeI vector to replace the 143bp that were removed to mutate the SalI site (no aa changes), creating pT7Blue2.XbaI-BlnIΔsal.

The 5' XbaI-BlnI (from pT7Blue2.XbaI-BlnI ΔSal) and the 3' BlnI-SalI (from pT7Blue2 BlnI-SalI) spike glycoprotein inserts were gel-purified and ligated them into the p893-1 XbaI-SalI vector (a vector derived from pLitmus 38 (New England Biolabs) with the alpha-factor leader sequence cloned into the BamHI -SalI sites of the MCS). The resulting full-length SARS Spike coding sequence was named p893-1.SARS Spike 1255 #9 (Figure 58).

E.coli HB101 competent cells were transformed with the oligo replacement ligation product and plated on Luria agar plates, containing 100μg/ml ampicillin. The desired clones were identified using miniscreen DNA analysis. After sequence verification of the positive clones, pT7Blue2 Xba-Bln ΔSal was chosen for use as a template for PCR reactions to amplify the Spike S1 1967 bp Xba-Sal fragment. The fragment was then subcloned into the p893-1 Xba-Sal vector, sequence verified, and named it p893-1.Spike S1 #11 (Figure 59).

In order to clone into the *S.cerevisiae* expression vector, pBS24.1, the 5' end of the S1 sequence had to be modified from XbaI to HindIII to allow ligation with the 3' HindIII end of the ADH2/GAPDH BamHI-HindIII promoter fragment. From pT7Blue2 Xba-BlnΔSal (described above) an AgeI-SalI 1943bp fragment was gel-purified. This fragment was ligated along with a

synthetic pair of HindIII-AgeI 30bp kinased oligos (S1-1+S1-2 creating the necessary 5' HindIII site) into the pSP72 HindIII-SalI commercial subcloning vector (named pSP72.SARS Spike S1 #2; Figure 59). S1-1 had SEQ ID NO: 9795 and S1-2 has SEQ ID NO: 9796.

After sequence verification of the positive clone from miniscreen DNA analysis, the HindIII-SalI fragment was gel purified. The 1365 bp BamHI-HindIII ADH2/GAPDH promoter fragment was ligated along with the 1973 bp HindIII-SalI S1 fragment into the pBS24.1 BamHI-SalI vector creating the genetically engineered pd.SARS Spike S1 #2 expression plasmid (Figure 60).

S.cerevisiae strain AD3 was transformed with pd.SARS Spike S1 #2 and single transformants were checked for expression after depletion of glucose in the medium. The recombinant protein was expressed at high levels in yeast, as detected by Coomassie blue staining. In particular, yeast cells were transformed with the SARS S1 expression plasmid using the Invitrogen S.c. EasyComp™ Transformation Kit. Expression in shown in Figure 57.

To express Spike 1195 protein, which does not contain the trans-membrane (TM) region or cytoplasmic tail that are present in the full-length SARS construct, the following series of genetic manipulations was performed:

From pT7Blue2 BlnI-SalI #11 (described above) a BlnI-DraI 1056bp fragment was gel purified. This fragment was ligated with a synthetic pair of 68bp DraI-SalI kinased oligos (DRS1+2; SEQ ID NOS: 9797 & 9798) into a pT7Blue2 BlnI-SalI vector (Figure 61). *E.coli* HB101 competent cells were transformed with the oligo replacement ligation product and plated on Luria agar plates, containing 100μg/ml ampicillin. The desired clones were identified using miniscreen DNA analysis. After sequence confirmation the clone was named pT7Blue2 BlnI-Sal Spike 1195 #7. The 1126bp BlnI-SalI fragment encoding the 3' end of the Spike 1195 was gel purified (Fig.61).

In order to generate the XbaI-SalI Spike 1195 fragment, the 3109bp XbaI-PciI fragment was isolated from the p893-1.SARS Spike 1255 #9 (described above) and a 457bp PciI-SalI fragment from pT7Blue2.SARS Spike 1195 #7 (described above). The two fragments were cloned into the p893-1 XbaI-SalI vector, creating the p893-1.SARS Spike 1195 #34 plasmid (Figure 62).

To clone SARS Spike 1195 into the pBS24.1 Saccharomyces cerevisisae expression vector, it was necessary to modify the 5' end of the SARS Spike 1195 from XbaI to HindIII, as done for the Spike S1 expression clone described above. To begin, the 2416bp AgeI-BlnI fragment was isolated from p893-1.SARS Spike 1195 #34. This fragment was ligated with the synthetic HindIII-AgeI 30bp oligos (described above to generate the S1 protein for expression in *S.cerevisiae*) into the pT7Blue2 HindIII-BlnI vector. E. coli HB101 competent cells were transformed with the oligo replacement ligation product and plated on Luria agar plates,

containing 100μg/ml ampicillin. The desired clones were identified using miniscreen DNA analysis. After sequence verification of the positive clone and plasmid amplification of pT7Blue2.SARS 1195 5' HindIII-BlnI #10 (Figure 63), we isolated a 402bp HindIII-NcoI fragment and the 2044bp NcoI-BlnI fragment (Figure 63). It was necessary for the HindIII-BlnI isolation to be done in two steps to avoid cloning issues related to the internal HindIII site located at nucleotide number 1319 of the spike 1195 protein.

To assemble the BamHI-SalI-expression cassette of Spike 1195 into the pBS24.1 vector *E.coli* HB101 competent cells were transformed with the the BamHI-HindIII (ADH2/GAPDH promoter), HindIII-NcoI 402bp fragment, NcoI-BlnI 2044bp and the BlnI-SalI 1126bp fragments into the pBS24.1 BamHI-SalI vector. The samples were plated on Luria agar plates, containing 100μg/ml ampicillin. The desired clone was identified using miniscreen DNA analysis, thus creating the genetically engineered pd.SARS Spike 1195 #10 (Figure 64).

S.cerevisiae strain AD3 was transformed with pd.SARS Spike 1195 #10 and single transformants were checked for expression after depletion of glucose in the medium. The recombinant protein was detected by Coomassie blue staining. In particular, yeast cells were transformed with the SARS 1195 expression plasmid using the Invitrogen S.c. EasyCompT™ Transformation Kit.

EXAMPLE 23: Expression in mammalian cell lines

cDNA fragments containing the S protein ORF of 1255 amino acids were amplified by RT-PCR from SARS viral RNA (Frankfurt isolate) grown in Vero cells. The amplified PCR fragments were cloned into pBlueScript vector, sequenced, and consensus spike sequence was assembled to create a full-length SARS spike clone, pBSnSh. *In vitro* transcription of pBSnSh followed by translation in a rabbit reticulocyte lysate resulted in the production of single polypeptide with an estimated molecular mass of ~140 kDa.

The insert of this plasmid was recloned via XhoI and Not I into a mammlian expression vector pCMVIII (Srivastava *et al.* (2003) *J. Virol.* 77:11244-11259) to create a construct, nSh (Fig. 74A). A PCR fragment containing a spike protein of 1195 amino acid, which was deleted for transmembrane (TM) domain and cystein-rich cytoplasmic tail (Cy) was amplified and cloned pCMVIII vector to generate the contstruct nShΔTC (Figure.74B). Both constructs were tagged with six histidine residues at the C-terminus in order to aid in their characterization. The Xho I/Not I fragment without a histidine tag also was subcloned into the alphavirus replicon vector backbone pVCRchim2.1 for use in the production of an alphavirus replicon particle chimera that expresses S protein. Production and characterization of the replication defective alphavirus vector particles was performed essentially as described previously (Perri *et al.* (2003)

J. Virol. 77:10394-10403; Polo *et al.* (1999) *PNAS USA.* 96:4598-4603). The resultant alphavirus vector particles were named as VEE/SIN.

COS7 cells and BHK-21 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum at 37°C and 5% CO_2 in air. COS7 cells were transfected with expression plasmids (nSh, nShΔTC) using a transfection kit (TransIt-COS, Mirus) following the manufacturer's protocol. The cells were washed once with ice-cold PBS and lysed with 1x Lysis buffer (20mM MOPS, 10mM NaCl, 1.5mM $MgCl_2$, and 1% Triton X-100) containing complete mini protease inhibitor (Roche). After a 30-min incubation on ice, the debris was cleared by centrifugation. The cleared lysate was either purified or used directly in western blotting.

To purify secreted spike proteins, medium from transfected cells was collected and subjected to centrifugation at 12,000 rpm for 10 min to remove cellular debris. The cleared medium was applied to a ConA-agarose column (Vector Lab). The column was washed extensively with 20mM sodium phosphate buffer, and then the bound proteins were eluted with 1M methyl α-D-mannopyranoside (MMP), 1M NaCl in 20mM sodium phosphate buffer. Column fractions containing SARS-CoV spike proteins were applied to MagneHis Protein purification system (Promega) following the protocol suggested by the manufacturer.

For western blot analysis, proteins were separated by 4-20% SDS-PAGE and then transferred electrophoretically to nitrocellulose membrane (Invitrogen). Membrane was blocked in blocking buffer (5% skim milk and 0.1% Tween 20 in PBS) and incubated with indicated antibody at room temperature for 1 hr, washed and probed with horseradish peroxidase (HRP)-conjugated secondary antibody (Biosource) followed by chemiluminescence (ECL system, Amersham) and exposed by X-ray films. The antibodies used were a mouse monoclonal anti-histidine antibody (anti-His•tag Mab, Novagen), a rabbit polyclonlal antipeptide antibody against SARS-CoV spike proten (SmPab, Abgent), or rabbit anti-SARS sera (2BE) obtained by immunization of rabbits with purified SARS-CoV virion. The latter has a cell culture neutralizing titer of 1/2,500. Unless stated otherwise, antibody was used at 1/1,000 for anti-histidine antibody and SmPab and 1/10,000 for anti-SARS rabbit sera.

Some spike proteins were treated with Peptide-N glycosidase F (PNGase F). Cell lysates were diluted in 0.5% SDS and 1% β-mercaptoethanol and denatured at 100°C for 10 min. After 2-fold dilution with 1% NP-40 in 50mM sodium phosphate (pH 7.5), the samples were treated with PNGase F (NEB) at 37°C for 1 hr. Enzyme-treated samples were analyzed by 4-12% SDS-PAGE in reducing condition. For a partial digestion with the PNGase, the cell lysates were diluted with 50mM sodium phosphate (pH 6.0) containing 0.75% Triton-X and treated with PNGase F (Calbiochem) at 37°C for 3 hr. Enzyme-treated samples were analyzed by 4-20% SDS-PAGE in nonreducing condition.

Western blots of cells 48-hours after transfection are shown in Figure 75. The S protein was detected in cell lysates as a doublet with estimated molecular weight of ~170 ~180 kDa , when the lysate was boiled and analysed under reducing SDS-PAGE conditions (Fig. 75A, lane 3). This doublet appears to result from differential glycosylation of one polypeptide product since pre-treatment of the cell lysate with PNGase F reduced the doublet to a single species of ~140 kDa (Fig. 75A, lane 4). This is the expected size predicted from the aa sequence for a full-length, intact polypeptide product. This experiment indicates that the full length SARS-CoV S is expressed in mammalian cells as a single, uncleaved polypeptide, but in two differentially glycosylated forms, gp170 and gp180 respectively. Unlike the two S glycoforms encoded by the full-length sequence, none of which were secreted, the SΔ protein product was detected both in cell lysates (Fig. 75A, lane 5) as well as in the cell culture medium (Fig. 75B, lane3) as a single species of ~ 160 kDa.

In order to further characterize the intracellular processing of the S protein, and as described above, BHK21 cells were infected with defective alphavirus particles expressing the full-length S. At 6 hr post infection with a MOI of 5, infected cells were pulse labeled for 1 hr with L-[^{35}S] methionine/cysteine and chased for 2 or 4 hours. The [^{35}S]-labeled S protein was immuno-precipitated using the rabbit antiserum raised against inactivated, purified virus and then digested with Endo H. The Endo H treatment involved dilution with a sample buffer (50mM sodium phosphate, 0.1% SDS, 50 mM DTT, pH 6.0) and boiling for 5 min. After denaturation, the samples were further diluted with 0.75% Triton-X 100 and treated with endoglycosidase H (Endo H) following manufacturer's protocol (Calbiochem) for 3 hr at 37°C. Enzyme-treated samples were added with gel loading buffer containing 0.1% SDS and DTT and analyzed by 8% SDS-PAGE.

Both digested and undigested proteins were boiled in SDS and analysed by reducing SDS-PAGE (Figure 55). After a 1-hr pulse, the S protein was apparent as a single gp170 component that was Endo H sensitive (lanes 1 and 2). After a 2-hr chase, a new species (gp180) was present along with gp170 in approximately equal proportions (lane3). After a 4-hr chase, the gp180 species was the dominant S protein component (lane 5) that was now Endo H resistant (lanes 5 and 6). This data is consistent with gp170 being an ER-resident glycoprotein containing high mannose chains and with gp180 corresponding with a Golgi-processed glycoprotein containing Endo H-resistant complex oligosaccharides.

The Endo H sensitivity of the C-terminus deleted SΔ protein purified from cell culture media ws also tested. As shown in Figure 76, the SΔ observed within cell lysates was found to be Endo H sensitive (lanes 1 and 2), while the secreted SΔ in cell culture media was Endo H resistant (lanes 3 and 4). This result is consistent with this glycoprotein being synthesized in an

immature form in the ER prior to transfer to the Golgi where the complex carbohydrate is added and the protein then secreted.

As already described, the S protein expressed in COS7 cells was detected as a gp170/gp180 doublet in western blot analyses of cell lysates that were fully denatured by boiling in the presence of DTT. However, the majority of S protein was detected as a high molecular glycoprotein in the 440-669 kDa range when the same cell lysate was not heat-denatured prior to western blot analysis using SDS-PAGE (Fig. 77, lane 1). The ~500 kDa species was resistant to 10 mM DTT treatment (lane 3) and not dissociated into the monomeric form unless the lysate was first heat-denatured at 100°C (lane 4). In contrast, oligomeric form of a test protein (Thyroglobulin) of which quaternary structure is held by disulfide-linkage was converted into subunit form by the 10 mM DTT treatment. These data suggest that the ~500 kDa oligomeric form of S protein is not disulfide-linked and is heat labile. To confirm the heat-sensitivity of the ~500 kDa species of S protein, the heat-denaturation experiment was repeated but without DTT. As shown in Figure 78, heat denaturation of ~500 kDa protein at 100°C alone was sufficient to convert it into gp170/180 monomeric forms (lane 4). Using a 80°C heat-denaturation step, both the ~500 kDa and monomeric forms were detectable in similar proportion (lane 3).

In order to investigate further whether this ~500 kDa species represents an S protein oligomer in native conformation, comparative analyses with virion-derived S glycoprotein derived from Vero cell cultures was performed. The purified virions were solubilised in 1% SDS prior to Western blot analyses after SDS PAGE. The presence of the ~500 kDa spike protein oligomer was confirmed in virion particles (Fig. 79, lane 1). In addition, heat denaturation of solubilised virions produced the same oligomer-to-monomer conversion as seen with the full-length recombinant S (lanes 2,3). The oligomeric nature of virion S was further analysed in a cross-linking experiment. Aliquots of inactivated virion from sucrose gradient fractions were treated with 10% SDS at 1% final concentration and diluted 2-fold with 0.2M Triethanolamine-HCl (pH 8, Sigma); Dimethyl suberimidate (DMS; Pierce Chemical Co.) was then added from a freshly prepared solution (10mg/ml in 0.2M Triethanolamine-HCl) at 3.3mg/ml final concentration. After 2 hr at room temperature, samples were concentrated with Centricon-30 and analyzed by silver staining after electrophoresis on a 4% polyacrylamide gel. Both untreated and DMS cross-linked virion proteins were heat-denatured, and the heat effect on the maintenance of oligomer structure was analysed by SDS-PAGE and silver staining (Figure 80). In the absence of cross-linking, heat denaturation resulted in the replacement of the ~500 kD spike protein species with the monomer species. In contrast, in the cross-linked proteins, the levels of the ~500 kD and monomer species did not change significantly after heating. These data support the fact that the ~500 kD protein is an oligomer of S monomer proteins that are bound non-covalently. After cross-linking and boiling, the ~500 kDa species migrated as a somewhat slower diffuse form

than the untreated form. This mobility shift is probably due to a structural change resulting from boiling. In addition, a minor protein species of ~300 kD, which may represent a non-dissociated S dimer, could be seen.

To estimate more precisely the size of the recombinant ~500 kDa S species expressed in COS7 cells, a COS7 cell lysate containing the S protein oligomer was fractionated using size-exclusion column chromatography. The major portion of the ~500 kDa oligomer co-eluted with a 572 kDa marker protein. Taken together, these experiments suggest that the ~500 kDa S species seen in COS7 cell lysates is probably a homotrimer of the S protein monomer.

The oligomeric status of the SΔ spike protein was also examined after expression in COS7 cells. As shown in Figure 81, the recombinant SΔ proteins present in cell lysates were also detected in high molecular weight forms of ~500 kDa range when the lysate was not heated prior to SDS-PAGE and Western blot analysis (lane 1). However, the efficiency of oligomerization by intracellular SΔ protein appears to be much less (<10%) compared to that of full-length S protein under the same western analysis conditions. A heat-sensitivity test on this ~500 kDa protein showed that the SΔ oligomer was more heat labile than that of the full-length S oligomer, as demonstrated by the >90% conversion of all of the ~500 kDa species into monomeric Sd forms at 80°C (lane 2). Also (Figure 82), the majority of the secreted SΔ protein was found in monomeric form with the ~500 kDa species barely detectable (and only detectable when the protein was loaded in excess for Western analysis) (lane 1). At a temperature above 80°C, all secreted SΔ proteins were detected as monomers (lanes 2, 3).

The ~500kDa protein is glycosylated, and the effect of deglycosylation on its antibody binding was investigated. The recombinant COS7 lysate was treated with PNGase F under non-denaturing condition (as described above) and analysed by western blot. As shown in Figure 83, deglycosylation did not affect the binding of anti-histidine Mab antibody to the treated S oligomer (lanes 2,3). However, it compromised the reactivity with the rabbit antisera raised against purified virus (lane 6). This antiserum binds to virion-derived S in western blot analyses only when DTT is omitted from the sample for SDS-PAGE indicating that it recognizes primarily a discontinuous, conformational epitope(s). This antisera has also been shown to have a high-titer of viral neutralizing antibodies. Its lack of binding to deglycosylated, recombinant S suggests that the carbohydrate actively contributes to the higher order, native structure of the S polypeptide oligomer.

The difference between the recombinant S and SΔ protein is the presence or absence of the TM-and Cys-rich domains at the C-terminus. This difference predicts that full-length S would be found associated with the membrane fraction while Sd would be in the soluble fraction upon lysis of transfected cells. Therefore, nSh- or nShΔTC-transfected cells were lysed under hypotonic conditions and the soluble cytosol fraction was separated from the insoluble

membrane fraction by centrifugation (Figure 48). As shown in Figure 84, the S protein was found in the membrane fraction (DF) both as a ~500 kDa and 180/170 kDa species (lane 4) but was not detectable in the soluble cytosol fraction (AF) (lane 3). However, the truncated SΔ protein was found as a monomeric species (gp170) in both fractions (lanes 5,6). This indicates that the C-terminal TM and Cys-rich domains are required for the anchorage of the S protein to cell membrane.

The cellular location of the S and SΔ proteins in COS7 cells was analyzed by indirect immunofluorescence microscopy. At 48 hr post-transfection, cells were directly fixed with 2% paraformaldehyde without detergent for cell surface staining or treated with detergent followed by Cytofix/Cytoperm solution for intracellular staining. Fixed cells were then stained with rabbit anti-SARS sera (2BE) and FITC-conjugated antibody. The nSh-transfected cells showed foci of S protein indicative of Golgi-localisation (Figure 85A), while the nShΔTC-transfected cells displayed a uniform distribution of SΔ protein throughout the cytoplasm indicative of ER localisation (Figure 85B). While the complete S protein was also observed on the surface of transfected cells in unfixed cells (Figure 85D), the SΔ was undetectable on the cell surface (Figure 85E). These results indicate the role played by the TM-and Cys-rich domains in anchoring the S protein to the plasma membrane. Although the TM-region alone is likely responsible for membrane anchorage, the potential role played by the Cys-rich region remains to be determined.

The SARS recombinant full-length S protein is thus an N-linked glycoprotein with an estimated molecular weight of 170-180,000 kDa. Deglycosylation with PNGase F resulted in a polypeptide of the expected size for the uncleaved, encoded polypeptide (140kDa). Both transient and stable expression of the full-length SARS-CoV S gene in a variety of mammalian cells, including COS7, 293, BHK21, and Huh7 cell lines, consistently produced a S protein doublet (gp170/180) as detected in western blot analyses. Pulse-chase analyses of transfected cells demonstrated that the SARS CoV S protein was initially synthesized as an Endo H sensitive gp170 species followed by the gradual appearance of an Endo H resistant gp180 form, presumably as a result of the addition of complex carbohydrate within the Golgi apparatus.

The recombinant S protein was not secreted into the cell culture medium unless the C-terminal 60 amino acids containing the TM-region and the Cys-rich tail were deleted.

The quaternary structure of the full-length recombinant S protein was investigated using cross-linking treatment, heat-denaturation, and size fractionation analyses. The results data are consistent with the recombinant S protein existing as a homotrimer of ~500kDa. Similar analyses of virion-derived S yielded the same results. Such a trimeric structure has been reported for other enveloped RNA viruses: the hemagglutinin HA of influenza virus, the E1-E2 heterodimer of alphaviruses and the G protein of vesicular stomatitis virus. Incubations under reducing

conditions indicate that the SARS-CoV S trimeric structure is non-covalently associated, and is very stable. S oligomers present in the cell lysate were shown to be resistant to reduction by 10 mM DTT, detergent treatment with 1% SDS, and heat denaturation at up to 60°C . Incubation at a temperature higher than >80°C resulted in the dissociation of the trimeric complex as evidenced by the decrease in trimer with the concomitant increase in the monomer bands. The temperature-induced appearance of the high-mannosylated gp170 (ER monomer form) as well as the complex-glycosylated gp180 (Golgi monomer form) suggests that trimerization can occur before the transport of the monomer spike protein to the medial Golgi apparatus. This is consistent with other reports for TGEV, influenza virus HA, and vesicular stomatitis virus G proteins. With these proteins, trimerization was reported to take place before addition of complex oligosaccharides in the Golgi apparatus.

The C-terminally truncated form of S was found in the cell lysate in both oligomeric and monomeric forms at a frequency of 10% and 90%, respectively. The truncated protein secreted into medium was found fully glysosylated and it was essentially all in monomeric form. We conclude that the C-terminal 60 amino acids of the S glycoprotein contains a membrane anchor region that affects the efficiency of trimerization. In S protein trimerization, it is possible that the C-terminal region is required to initiate the event and the triple-stranded coiled coil structures in the S2 stalk domain provide further stabilizing force as seen in HA oligomer of influenza virus.

EXAMPLE 24: CHO cells for Spike protein expression

CHO cell lines that stably express either the full-length or truncated SARS-CoV spike proteins were prepared. Several stably transfected CHO cell lines were obtained, and Figure 73 shows western blot data from a panel of representative clones.

EXAMPLE 25: Expression in E.coli

All SARS-CoV ORFs (Figure 17, Table 10) were cloned in the pET vector and expressed as C-terminal His-Tag fusion proteins in *E.coli*. The proteins smaller than 16KD were also expressed as N-terminal GST (Glutathione S-transferase) fusion proteins using pGEX vector.

Nsp1 and Nsp2, the two SARS-CoV proteins with proteolytic activity, were not expressed as full length proteins due to toxicity in *E.coli*. The respective genes were instead cloned in different portions in order to separate the catalytic residues (Cys833/ His994 for Nsp1; His41/Cys145 for Nsp2) in the resulting recombinant proteins: Nsp1A from nucleotides 2719-5214 of AY310120; Nsp1B from nucleotides 5218-7371; Nsp1C from nucleotide 7372-9984; Nsp2A from nucleotide 9985-10416; Nsp2B from nucleotide 10476-10902.

Nsp9 (SEQ ID NO: 9775) was divided into two portions: Nsp9A from nucleotide 13371- 14756; Nsp9B from nucleotide 14757-16166.

Matrix (M), ORF3 and ORF7 contain respectively three, two and one transmembrane domains. These proteins were expressed as deletion proteins excluding the first 100 amino acids (M and ORF3) or the first 18 amino acids (ORF7) that include the hydrophobic regions.

The cloned sequences are shown in Table 26.

A two-step strategy was used to amplify the cloned sequences. In the first step, amplification of DNA fragments containing more than one gene or single gene used sequenced cDNA as template. Eleven cDNA sequences were amplified: (1) a fragment, named amplC1, including genes coding for protein E, protein M, orf 7-8-9-10; (2) a fragment, named amplC2, including genes coding for orf 3-4; (3) a fragment, named amplC5, including genes coding for proteins Nsp12 and Nsp13; (4) Nsp11gene; (5) P28 and P65 genes; (6) Nsp1B and Nsp1C genes portion; (7) a fragment, named amplC9, including genes coding for proteins Nsp2 and Nsp3; (8) a fragment, named amplNsp4-7, including genes coding for proteins Nsp4, Nsp5, Nsp6, Nsp7 and for amplification of Nsp9A gene portion; (9) Nsp 9B gene portion and Nsp10 gene; (10) a fragment, named amplCO, including genes coding for proteins Orf11, Nucleocapsid (N) and Orf12; (11) Nsp1A gene portion. The primers used in this first step are given in Table 27:

In the second step, amplification of single genes was performed using DNA fragments from the first amplification step as templates. The primers are shown in Table 28.

Of the proteins where expression was seen, it was either in inclusion bodies (insoluble) or in a soluble form. Purification proceeded on appropriate material. Table 29 shows the molecular weight of the expressed fragments of SARS-CoV ORFs, whether they were cloned (+ or –), whether the cloned fragment was seen to be expressed (+ or –) and the form of protein which was chosen for purification.

Where a protein was a soluble His-tagged product, a single colony was streaked and grown overnight at 37°C on a LB/Amp (100 μg/ml) agar plate. An isolated colony from this plate was inoculated into 20ml of LB/Amp (100 μg/ml) liquid medium and grown overnight at 37°C with shaking. The overnight culture was diluted 1:30 into 1.0 L LB/Amp (100 μg/ml) liquid medium and allowed to grow at the optimal temperature (30 or 37°C) until the OD550nm reached 0.6-0.8. Expression of recombinant protein was induced by addition of IPTG (final concentration 1.0 mM) and the culture incubated for a further 3 hours. Bacteria were harvested by centrifugation at 8000 x *g* for 15 min at 4°C. The bacterial pellet was resuspended in 10 ml of cold buffer A (300 mM NaCl, 50 mM phosphate buffer, 10 mM imidazole, pH 8.0). Cells were disrupted by sonication (or French Press) on ice four times for 30 sec at 40W using a Branson sonifier 450 and centrifuged at 13 000xg for 30 min at 4°C. Supernatants were mixed with 150μl Ni^{2+}-resin (previously equilibrated with buffer A) and incubated at room temperature with gentle agitation for 30 min. The resin was Chelating Sepharose Fast Flow (Pharmacia), prepared according to the manufacturer's protocol. The batch-wise preparation was centrifuged at 700 x *g* for 5 min at 4°C

and the supernatant discarded. The resin was washed twice (batch-wise) with 10ml buffer A for 10 min, resuspended in 1.0 ml buffer A and loaded onto a disposable column. The resin continued to be washed with buffer A at 4°C until the OD280nm of the flow-through reached 0.02-0.01. The resin was further washed with cold buffer B(300 mM NaCl, 50 mM phosphate buffer, 20 mM imidazole, pH 8.0) until the the OD280nm of the flow-through reached 0.02-0.01. The His-fusion protein was eluted by addition of 700μl of cold elution buffer C (300 mM NaCl, 50mM phosphate buffer, 250 mM imidazole, pH 8.0) and fractions collected until the OD_{280nm} indicated all the recombinant protein was obtained. 20μl aliquots of each elution fraction were analyzed by SDS-PAGE. Protein concentrations were estimated using the Bradford assay.

Where a protein was seen as an insoluble product, the inclusion bodies were purified as follows: homogenize cells (5 g wet weight) in 25 ml 0.1M Tris HCl pH 7, 1mM EDTA, at 4°C using an ultraturrax (10000 rpm); add 1.5mg lysozyme per gram cells; mix shortly with an ultraturrax and incubate at 4°C for 30'; use sonication or high-pressure homogenization to disrupt the cells; to digest DNA, add $MgCl_2$ to a final concentration of 3mM and DNase to a final concentration of 10ug/ml and incubate 30' at 25°C. add 0.5 vol of 60mM EDTA, 6% Triton x-100, 1.5M NaCl pH 7.0 to the solution, and incubate for 30' at 4°C; centrifugation at 31000 g for 10' at 4°C; re-suspend pellet in 40ml of 0.1M Tris HCl pH 7.0, 20 mM EDTA using ultraturrax; centrifugation at 31000 g for 10' a 4°C; store the IB pellet at – 20°C.

The results of expression are shown in Figures 86 to 105. Examples of purity and yield are given in Table 30.

EXAMPLE 26: Retention of critical epitope on truncated Spike antigen

A human monoclonal antibody having neutralizing activity was tested in an ELISA assay for reactivity with the purified truncated Spike protein. Briefly, ELISA plates were coated with truncated form of the spike protein at a concentration of 1μg/ml (100μl/ well) by incubating the plates overnight at 4°C. The plates were washed, non-specific binding sites were blocked and then different dilutions of the antibody were added and plates were incubated for 1 hour at room temperature. At the end of incubation, the plates were washed and bound antibody was detected by using anti-human IgG conjugated to horse radish peroxidase (HRP) and an appropriate substrate. The optical density of each well was recorded at 405 nm using an ELISA reader. The data are shown in Figure 69 and clearly demonstrate that the neutralizing epitope recognized by the mAb is preserved and exposed on the recombinant truncated Spike protein.

EXAMPLE 27: Different Spike vaccines

Purified truncated spike protein was used to immunize mice and the level of binding antibodies induced against the truncated spike protein was determined by ELISA assay. Briefly a group of 10 mice were immunized with 3μg of truncated spike protein adjuvanted in MF59 at 0,

4 and 8 weeks intervals. Sera samples were collected from these animals and assayed for antibodies induced by truncated spike protein in an ELISA assay. An additional group of 8 mice was immunized with 75 μg of DNA encoding the truncated form of the spike protein on PLG particles at 0, 4 and 13 weeks intervals, the sera were collected and analyzed as above for anti-spike antibodies as above

The profile of binding antibodies induced in each group was plotted as geometric mean titer (GMT). Compared to a plasmid DNA vaccine expressing truncated spike antigen and delivered using a PLG microparticle formulation, the purified truncated spike protein was significantly more potent for inducing strong antibody responses. Further comparison with the antibody responses induced by inactivated BPL-SARS-CoV (already shown protective) in the same mouse strain revealed that the magnitude of antibody responses induced by purified truncated spike protein and the inactivated virus vaccine are in the same range (Figure 70).

The neutralization potential of antibodies induced by the recombinant truncated spike protein, or plasmid DNA expressing the same spike antigen, were also evaluated. The GMT values obtained in both groups are shown in Figure 71. From these data, it appears that the purified protein is significantly more effective at inducing neutralizing antibody responses against the SARS-CoV spike. Furthermore, the neutralization titers typically induced by the purified truncated spike protein are comparable to neutralization titers induced by an inactivated SARS-CoV vaccine.

Figure 72 shows a comparison of antibody binding levels (ELISA, X-axis) with neutralization titers (Y-axis). In general there is a very good correlation between the binding and neutralizing antibodies. The bottom-left grouping shows ratios 2 weeks post-3rd immunization with the DNA vaccine; the top-right grouping shows ratios 2 weeks post-2nd immunization with the protein vaccine. Both forms of vaccine show a consistent correlation.

In further experiments, the ability of a DNA vaccine to invoke an immune response in mice was studied. Mice were immunized with pCMV-nSdTC plasmid, either free or with PLG microparticles. Serum from the mice was then used as the staining antibody against cultured 293 cells that had been transfected with spike, either full-length or truncated. The cells were centrifuged prior to testing and the pellet was lysed. The antibody was tested against the culture supernatant and against the cell lysate. As shown in Figure 112, the mouse serum was able to detect spike protein in the lysate of cells that expressed full-length spike and in the supernatant of cells that expressed the truncated spike protein. Results were comparable to the staining seen when using rabbit serum that had been obtained after immunization with whole killed virus. Thus anti-spike antibodies can be induced by the use of DNA vaccination.

EXAMPLE 28: Expression cassettes in pCMV

The sequence of plasmid pCMVKm2 is given as SEQ ID NO: 9923. Genes encoding the spike protein either in full-length form (pCMVKm2 SARS Spike nS; SEQ ID NO: 9921) or in its ΔTC form (pCMVKm2 SARS Spike nSΔTC; SEQ ID NO: 9922) were inserted into this basic vector.

Mice were immunized with these vectors, and with similar vectors encoding the N, M or E proteins. Vectors encoding the same proteins but with optimized codon usage were also prepared. Codons were optimized for efficient human expression starting from the FRA sequence (GenBank: AY310120). The optimized sequences are: N (SEQ ID NO: 9924); M (SEQ ID NO: 9925); E (SEQ ID NO: 9926).

After administration, expression of proteins could be detected by immunofluorescence in all cases. For example, Figure 106 shows immunofluorescence (using anti-SARS rabbit serum) results after administration of the vector encoding optimsed N antigen, revealing high level expression. Mice receiving the control vector alone showed no fluorescence.

Figure 107 compares immunofluorescence (using Abgent anti-M antibody) of the native M sequence (107A) or the codon-optimsed M sequence (107B). Similarly, Figure 108 compares immunofluorescence (using Abgent anti-E antibody) of the native E sequence (108A) or the codon-optimsed E sequence (108B).

Four groups of mice (8 mice per group) were immunized with: (1) SARS nS Spike, nSdTC truncated Spike, and N proteins; (2) pCMV-SARS-nSdTC: DNA+DNA-PLG at weeks 0,4 and 13 wks; (3) CMV-nS: DNA+DNA-PLG+VEE/SIN Rep at 0, 4 and 9 wks; (4) VEE/SIN Rep-SARS-nS three times at 0, 4 and 13 wks. Sera from all groups recognized SARS nS and nSdTC proteins, and also showed virus binding and neutralization activity.

EXAMPLE 29: Spike protein cleavage

To investigate the effect of proteolytic cleavage on SARS-CoV Spike protein, it was expressed in various forms in *E.coli*, including: (1) full-length S1-S2; (2) S1 alone; (3) HR1 heptad; and (4) HR2 heptad. The expressed proteins were used to raise immune rabbit sera, which were then used for visualizing western blots of Vero cells, either infected or not infected with SARS-CoV.

Figure 109 shows a western blot using a 1:10000 dilution of antibody raised against either the S1 domain or the uncleaved S1-S2 domains. Figure 110 shows a western blot using a 1:10000 dilution of antibody raised against each of the four proteins. The difference in antigen reactivity is readily apparent.

Figure 111 shows similar data. Each serum was tested against four lanes, with those gour lanes being from left to right: (a) serum at 1:500 dilution, SARS-CoV-infected cells; (b) serum at

1:500 dilution, non-infected cells; (c) serum at 1:2500 dilution, SARS-CoV-infected cells; (d) serum at 1:2500 dilution, non-infected cells. Again, the difference in antigen reactivity is readily apparent.

Figures 109-111 show that the Spike protein exists in various forms in infected Vero cells, with sizes of approx. 75kDa, 90kDa, 180kDa and >250kDa. The Spike protein is cleaved (at least partially) either intracellulary or after release of the particles.

If enzymatic cleavage of the mouse hepatitis coronavirus spike protein is inhibited then cell-cell fusion (syncytia formation) is also inhibited, but virus-cell fusion is not (de Haan *et al.* (2004) *J Virol*). Syncytia are observed *in vivo* in the lungs of SARS-infected patients, but are not seen in Vero cell cultures of the SARS-CoV. Inhibition of Spike protein cleavage may thus be used to prevent syncytia formation and related pathology, even though viral infectivity may not be blocked.

Example 30: Purification of SARS protease

Cells were grown at 37°C to mid-log phase and induced with 0.2% L-arabinose. Cells were harvested by centrifugation, and the cells resuspended in lysis buffer (LB) containing 20 mM Tris pH 7.5, 500 mM NaCl, 5% glycerol V/V, 0.05% Triton X-100, 5 mM βME, 5 mM imidazole, and complete protease inhibitors (–)EDTA. Benzonase was added to a final concentration of 50U/ml of lysate. Cells were then lysed using two passes through a pre-chilled microfluidizer. The lysate was clarified by high speed centrifugation at 44,000 x *g*. Clarified lysate was applied to a prepared Pharmacia chelating FF column charged with nickel sulfate. After application of the lysate the column was washed with 5 column volumes of LB, followed by 5 column volumes of LB supplemented with 45 mM imidazole. The column was then eluted using LB supplemented with 250 mM imidazole. Purity of the isolated SARS protease was 50%. Fractions containing protease were pooled, adjusted to 5 mM EDTA, and then applied to a Superdex 200 gel filtration column equilibrated in 20 mM Tris pH 7.5, 150 mM NaCl, 5% V/V glycerol, 0.05 % Triton X-100, and 5 mM DTT. Purity of the isolated SARS protease was 70%. Again, fractions containing the protease were pooled, and then stored at -80°C until used. Activity assay, mass spectrometry and western blot analysis were used to positively identify the protein (FIG 133). All steps were carried out with pre-chilled buffers, and kept at 4°C for as much of the preparation as possible.

Western of SARS Protease Purification Fractions

Protocol: Briefly, protein concentration was based on Absorbance at 280 nm, and coomassie stained gel estimates of purity. Protein was run on a 4-20% gradient gel, and transferred to nitrocellulose. The blot was then blocked with 3% BSA, probed with Mouse IgG anti-pentaHis,

and then probed with a secondary antibody to Mouse IgG conjugated with HRP. The blot was visualized using an ECL kit (Pharmacia Biotech). The results are shown in Figure 133 where A is the sizing column pool loaded at 50, 100 and 200 ng of target protein and B is the immobilized metal affinity column pool loaded at 50, 100 and 200 ng of target protein.

Example 31: Continuous Fluorescence Resonance Energy Transfer (FRET) Enzyme Assay

The peptide containing EDANS, the fluorescence donor, and DABCYL, the fluorescence quencher (DABCYL-*VNSTLQ ▽SGLRK*-EDANS) was synthesized by Syn. Pep. (Dublin, CA). The peptide contains the cleavage site Gln-Ser in the middle. Meyers, G. *et al.* Handbook of Proteolytic Enzymes and Barrett, A *et al.* Academic Press, London, 1998, 726-728. The proteolytic activity of SARS protease was followed kinetically by measuring the level of formation of cleaved product that contains the fluorescence donor, SGLRK-EDANS using the Hitachi fluorometer (F-4500 FL Spec.) set at 340 nm excitation and 490 nm emission wave length. 5 μL of 5 mM peptide stock in DMSO solution was added to the reaction mixture, containing 295 μl of standard buffer (75 mM Tris-Hcl, 25 mM NaOAc, 25 mM Bis-Tris, 25 mM glycine, 5 mM EDTA, and 1 mM EDTA, pH 7.4) and 100 ul of buffer or 100 ul of 3.6 uM protease stock solution. The kinetic curve was followed for 6 minutes (the reaction was linear with R2 value of 0.998 (FIG 134)). The formation of fluorescence (proteolytic reaction) is likely enzyme dependent, as concentration of enzyme was tripled three times as much fluorescence was formed in the 6 minutes time frame.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

Table 1. US Patents and Published International Patent Applications

Publication Number	Title	Publication Date
US-3927216	1,2,4-Triazol E-3-Carboxamides For Inhibiting Virus Infections	12/16/1975
US-4010269	Antiviral Quinazoline Compositions And Methods Of Use	3/1/1977
US-4065570	Antiviral 5-(Substituted Benzal) Hydantoins	12/27/1977
US-4089965	Thiazolylphenylguanidines As Antirhinovirus Agents	5/16/1978
US-4122191	Antirhinovirus Agents	10/24/1978
US-4192895	Antirhinovirus Agents	3/11/1980
US-4254144	Substituted Benzonitriles Having Antiviral Activity	3/3/1981
US-4264617	Antiviral 5-(Substituted Benzal) Hydantoins	4/28/1981
US-4287188	Purine Derivatives	9/1/1981
US-4327088	Phosphonooxy- Or Glycosyloxy-Substituted Acrylophenones, Compositions And Uses Thereof	4/27/1982
US-4332820	Substituted Benzonitriles Having Antiviral Activity	6/1/1982
US-4349568	Sulfur-Substituted Diphenyl Ethers Having Antiviral Activity	9/14/1982
US-4352792	3-Alkoxyflavone Antiviral Agents	10/5/1982
US-4371537	Sulfur-Substituted Phenoxypyridines Having Antiviral Activity	2/1/1983
US-4423053	Derivatives Of 2-Amino-5-(O-Sulphamidophenyl)-1,3,4-Thiadiazol As Antiviral Agents And A Process For The Preparation Thereof	12/27/1983
US-4505929	Sulfur-Substituted Diphenyl Ethers Having Antiviral Activity	3/19/1985
US-4526897	Hypertensive Isoindolin-2-Yl-Aminoimidazolines And Isoindolin-2-Yl-Guanidines	7/2/1985
US-4558134	Certain Phenoxy-Pyridine-Carbonitriles Having Antiviral Activity	12/10/1985
US-4629729	Endowed With Anti-Viral Activity 2-Alkylamino-4,6-Dihalo Pyrimidines	12/16/1986
US-4636492	Inhibition Of Viral Protease Activity By Peptide Halomethyl Ketones	1/13/1987
US-4652552	Tetrapeptide Methyl Ketone Inhibitors Of Viral Proteases	3/24/1987
US-4724233	Therapeutical Application Of Phosphonylmethoxyalkyl Adenines	2/9/1988
US-4738984	Antirhinovirus Agents	4/19/1988
US-4847246	Antiviral Compositions Derived From Fireflies And Their Methods Of Use	7/11/1989
US-4855283	Novel Pharmaceutically Active N-(2-Aminoacylamido-2-Deoxy-Hexosyl)-Amides, -Carbamates And -Ureas	8/8/1989
US-4885285	Phosphorus Compounds, Processes For Their Manufacture, And Their Use	12/5/1989
US-4956351	Antiviral Pharmaceutical Compositions Containing Cyclodextrins	9/11/1990
US-5001125	Anti-Virally Active Pyridazinamines	3/19/1991
US-5036072	Antiviral Agent	7/30/1991
US-5070090	Antipicorpaviral Herterocyclic-Substituted Morpholinyl Alkylphenol Ethers	12/3/1991
US-5100893	Antipicornaviral Pyridazinamines	3/31/1992
US-5112825	Antirhinoviral Heteroamine-Substituted Pyridazines	5/12/1992
US-5157035	Anti-Virally Active Pyridazinamines	10/20/1992
US-5240694	Combined Antiviral And Antimediator Treatment Of Common Colds	8/31/1993
US-5242924	Tetrazolyl-(Phenoxy And Phenoxyalkyl)-Piperidinylpyridazines As Antiviral Agents	9/7/1993
US-5278184	Synthetic Derivatives Of Pyrrole And Pyrrolidine Suitable For The Therapy Of Infections Caused By Rhinoviruses	1/11/1994
US-5364865	Phenoxy- And Phenoxyalkyl-Piperidines As Antiviral Agents	11/15/1994
US-5453433	Thiadiazoles And Antipicornaviral Compositions	9/26/1995
US-5492689	Combined Virustatic Antimediator (COVAM) Treatment Of Common Colds	2/20/1996
US-5514679	Therapeutic Phenoxyalklpyridazines And Intermediates Therefor	5/7/1996
US-5514692	Substituted Quinoline Derivatives Useful As Antipiconaviral Agents	5/7/1996
US-5523312	Antipicornaviral Agents	6/4/1996
US-5545653	Anti-Viral Compounds	8/13/1996
US-5552420	Therapeutic Phenoxyalkylazoles And Phenoxyalkylazines	9/3/1996
US-5567719	Thiadiazoles And Their Use As Antipicornaviral Agents	10/22/1996

US-5580897	1,2-Dithiins Having Antifungal Activity	12/3/1996
US-5618821	Therapeutic Phenoxyalkylheterocycles	4/8/1997
US-5618849	Orally Active Antiviral Compounds	4/8/1997
US-5648354	1,2-Dithiins Having Antifungal Activity	7/15/1997
US-5650419	Thiadiazoles And Their Use As Antipicornaviral Agents	7/22/1997
US-5693661	Anti-Viral Compounds	12/2/1997
US-5721261	Therapeutic Phenoxyalkylazoles And Phenoxyalkylazines	2/24/1998
US-5725859	Plant-Based Therapeutic Agent With Virustatic And Antiviral Effect	3/10/1998
US-5750527	Thiadiazoles And Their Use As Antipicornaviral Agents	5/12/1998
US-5750551	Treatment For Viral Diseases	5/12/1998
US-5762940	Methods And Compositions For Inhibiting Or Destroying Viruses Or Retroviruses	6/9/1998
US-5763461	Therapeutic Phenoxyalkylheterocycles	6/9/1998
US-5821242	Anti-Viral Compounds	10/13/1998
US-5821257	Thiadiazoles And Their Uses As Antipicornaviral Agents	10/13/1998
US-5821331	Anti-Picornaviral Agents	10/13/1998
US-5846986	Therapeutic Phenoxyalkylazoles And Phenoxyalkylazines	12/8/1998
US-5856530	Antipicornaviral Compounds And Methods For Their Use And Preparation	1/5/1999
US-5891874	Anti-Viral Compound	4/6/1999
US-5962487	Antipicornaviral Compounds And Methods For Their Use And Preparation	10/5/1999
US-6004933	Cysteine Protease Inhibitors	12/21/1999
US-6020371	Antipicornaviral Compounds Compositions Containing Them And Methods For Their Use	2/1/2000
US-6087374	Anti-Viral Compounds	7/11/2000
US-6114327	Anti-Viral Compounds	9/5/2000
US-6117844	Method And Composition For Antiviral Therapy	9/12/2000
US-6194447	Bis (Benzimidazole) Derivatives Serving As Potassium Blocking Agents	2/27/2001
US-6214799	Antipicornaviral Compounds And Methods For Their Use And Preparation	4/10/2001
US-6277891	Nitric Oxide Inhibits Rhinovirus Infection	8/21/2001
US-6294186	Antimicrobial Compositions Comprising A Benzoic Acid Analog And A Metal Salt	9/25/2001
US-6331554	Antipicornaviral Compounds, Compositions Containing Them, And Methods For Their Use	12/18/2001
US-6358971	Anti-Viral Compounds	3/19/2002
US-6362166	Antipicornaviral Compounds And Methods For Their Use And Preparation	3/26/2002
US-6414004	3-Substituted 5-Aryl-4-Isoxazolecarbonitriles Having Antiviral Activity	7/2/2002
US-6420591	Carbamates And Compositions Thereof, And Methods For Their Use For Treating Cancer, Inflammation, Or A Viral Infection	7/16/2002
US-6469018	Compounds	10/22/2002
US-6498178	Inhibitors Of IMPDH Enzyme	12/24/2002
US-6514997	Antipicornaviral Compounds And Compositions, Their Pharmaceutical Uses, And Materials For Their Synthesis	2/4/2003
US-6525043	Use Of Ion Channel Modulating Agents	2/25/2003
US-6531452	Antipicornaviral Compounds And Compositions, Their Pharmaceutical Uses, And Materials For Their Synthesis	3/11/2003
US-6534489	Organophosphorus Compounds And The Use Thereof	3/18/2003
WO00/06529	Diketoacid-Derivatives As Inhibitors Of Polymerases	2/10/2000
WO00/25791	Pyridin-4-Yl Or Pyrimidin-4-Yl Substituted Pyrazines	5/11/2000
WO00/27423	Methods And Compositions For Treating Common Cold Symptoms	5/18/2000
WO00/34308	Protein Transduction System And Methods Of Use Thereof	6/15/2000
WO00/39348	Methods And Compositions For Identifying Protease Modulators	7/6/2000
WO00/40243	Novel Compounds	7/13/2000
WO00/50037	Nitrosated And Nitrosylated Proton Pump Inhibitors, Compositions And Methods Of Use	8/31/2000
WO00/56331	Inhibitors Of Impdh Enzyme	9/28/2000
WO00/56757	Immunomodulatory Steroids, In Particular The Hemihydrate Of 16.Alpha.-Bromoepiandrosterone	9/28/2000
WO00/66096	New Indication For Use Of Antiepileptic Agents And Medicines	11/9/2000
WO00/78746	Antiviral Agents	12/28/2000

WO01/00199	Compounds Obtained From Salvia Species Having Antiviral Activity	1/4/2001
WO01/00585	Pyrazolidinol Compounds	1/4/2001
WO01/02551	Virus Like Particles, Their Preparation And Their Use Preferably In Pharmaceutical Screening And Functional Genomics	1/11/2001
WO01/03681	Use Of Flavones, Coumarins And Related Compounds To Treat Infections	1/18/2001
WO01/05396	Use Of Cobalt Chelates For Treating Or Preventing Virus Infection	1/25/2001
WO01/10894	Antipicornaviral Compounds And Compositions, Their Pharmaceutical Uses, And Materials For Their Synthesis	2/15/2001
WO01/19322	Use Of Csaids In Rhinovirus Infection	3/22/2001
WO01/19822	Antiviral Agents	3/22/2001
WO01/22920	Colon And Colon Cancer Associated Polynucleotides And Polypeptides	4/5/2001
WO01/25188	Novel Carbamates And Ureas	4/12/2001
WO01/31016	Processed Human Chemokines Phc-1 And Phc-2	5/3/2001
WO01/37837	3,4-Dihydro-(1h)-Quinazolin-2-Ones And Their Use As Csbp/P38 Kinase Inhibitors	5/31/2001
WO01/38312	3,4-Dihydro-(1h)Quinazolin-2-One Compounds As Csbp/P38 Kinase Inhibitors	5/31/2001
WO01/38313	3,4-Dihydro-(1h)Quinazolin-2-One Compounds As Csbp/P39 Kinase Inhibitors	5/31/2001
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WO01/40189	Antipicornaviral Compounds And Compositions, Their Pharmaceutical Uses, And Materials For Their Synthesis	6/7/2001
WO01/49303	Multivalent Electron Active Compositions And Methods Of Making And Using Same	7/12/2001
WO01/60393	Selective Destruction Of Cells Infected With Human Immunodeficiency Virus	8/23/2001
WO01/62726	2-Oxo-1-Pyrrolidine Derivatives, Processes For Preparing Them And Their Uses	8/30/2001
WO01/79167	Antipicornaviral Compounds And Compositions, Their Pharmaceutical Uses, And Materials For Their Synthesis	10/25/2001
WO01/90047	Novel Mmp-2/Mmp-9 Inhibitors	11/29/2001
WO01/90129	Prophylactic And Therapeutic Treatment Of Infectious And Other Diseases With Mono- And Disaccharide-Based Compounds	11/29/2001
WO01/92499	Nucleic Acid Molecules Encoding A Protein Interacting With Ser/Thr Kinase Akt	12/6/2001
WO01/93883	Therapeutic Agents - Iii	12/13/2001
WO01/93884	Therapeutic Agents - I	12/13/2001
WO01/93885	Therapeutic Agents - Ii	12/13/2001
WO01/96297	Antipicornaviral Compounds And Compositions, Their Pharmaceutical Uses, And Materials For Their Synthesis	12/20/2001
WO02/04413	Chiral Integrin Modulators And Methods Of Use Thereof	1/17/2002
WO02/11743	Treatment Of Prostate Cancer	2/14/2002
WO02/12477	Enhanced Replication Of Hcv Rna	2/14/2002
WO02/14343	Immunosuppressive, Antiinflammatory And Analgetic Compounds	2/21/2002
WO02/24145	Antiviral Substances From Plant Cuticular And Epicuticular Material	3/28/2002
WO02/28351	Recombinant Mucin Binding Proteins From Steptococcus Pneumoniae	4/11/2002
WO02/30442	Method For Treating Cytokine Mediated Hepatic Injury	4/18/2002
WO02/34771	Nucleic Acids And Proteins From Streptococcus Groups A & B	5/2/2002
WO02/44737	Diagnostic And Therapeutic Compositions And Methods Related To G Protein-Coupled Receptor (Gpcr) Anaphylatoxin C3a Receptor	6/6/2002
WO02/50045	Antiviral Agents	6/27/2002
WO02/51413	Macrocyclic Anti-Viral Compounds	7/4/2002
WO02/53138	Treatment For Inhibiting Neoplastic Lesions	7/11/2002
WO02/57425	Nucleoside Derivatives As Inhibitors Of Rna-Dependent Rna Viral Polymerase	7/25/2002
WO02/59083	Novel Compounds	8/1/2002
WO02/60875	Nicotinamide Biaryl Derivatives Useful As Inhibitors Of Pde4 Isozymes	8/8/2002
WO02/60898	Thiazolyl-, Oxazolyl-, Pyrrolyl-, And Imidazolyl-Acid Amide Derivatives Useful As Inhibitors Of Pde4 Isozymes	8/8/2002
WO02/69903	Nucleosides, Preparation Thereof And Use As Inhibitors Of Rna Viral Polymerases	9/12/2002
WO02/72022	Substituted Tetracycline Compounds As Antifungal Agents	9/19/2002

WO02/72031	Substituted Tetracycline Compounds As Synergistic Antifungal Agents	9/19/2002
WO02/76939	Cysteine Protease Inhibitors	10/3/2002
WO02/77021	Streptococcus Pneumoniae Proteins And Nucleic Acids	10/3/2002
WO02/79401	Novel Rgs9 Protein Binding Interactions And Methods Of Use Thereof	10/10/2002
WO02/82041	Production And Use Of Novel Peptide-Based Agents For Use With Bi-Specific Antibodies	10/17/2002
WO02/87465	Compositions And Methods Of Double-Targeting Virus Infections And Cancer Cells	11/7/2002
WO02/87500	Viral Enzyme Activated Prototoxophores And Use Of Same To Treat Viral Infections	11/7/2002
WO02/88091	Inhibitors Of Human Rhinovirus 2a Cysteine Protease	11/7/2002
WO02/89832	Pharmaceutical Compositions For Preventing Or Treating Th1 And Th2 Cell Related Diseases By Modulating The Th1/Th2 Ratio.	11/14/2002
WO02/92779	Method For Enriching Tissues In Long Chain Polyunsaturated Fatty Acids	11/21/2002
WO02/94185	Conjugates And Compositions For Cellular Delivery	11/28/2002
WO02/94868	Staphylococcus Aureus Proteins And Nucleic Acids	11/28/2002
WO02/96867	Inhibitors Of Protein Kinase For The Treatment Of Disease	12/5/2002
WO02/98424	Novel Anti-Infectives	12/12/2002
WO03/04489	Compositions And Methods For Inhibiting Prenyltransferases	1/16/2003
WO03/08628	Enzymatic Nucleic Acid Peptide Conjugates	1/30/2003
WO03/15744	Chitin Microparticles And Their Medical Uses	2/27/2003
WO03/20222	Dioxolane And Oxathiolane Derivatives As Inhibitors Of Rna-Dependent Rna Viral Polymerase	3/13/2003
WO03/20270	Oxadiazolyl-Phenoxyalkylisoxazoles, Compositions Thereof And Methods For Their Use As Anti-Picornaviral Agents	3/13/2003
WO03/20271	Oxadiazolyl-Phenoxyalkylisoxazoles, Compositions Thereof And Methods For Their Use As Anti-Picornaviral Agents	3/13/2003
WO03/20712	Oxadiazolyl-Phenoxyalkylthiadiazoles, Compositions Thereof And Methods For Their Use As Anti-Picornaviral Agents	3/13/2003
WO86/03412	Improvements Relating To The Treatment Control And Prevention Of Rhinovirus Infections	6/19/1986
WO86/03971	Antiviral Agents	7/17/1986
WO88/09669	Avirulent Microbes And Uses Therefor	12/15/1988
WO92/03475	Enterovirus Peptides	3/5/1992
WO92/22520	Orally Active Antiviral Compounds	12/23/1992
WO92/22570	Inhibitors Of Picornavirus Proteases	12/23/1992
WO94/00012	Nucleic Acids And Methods Of Use Thereof For Controlling Viral Pathogens	1/6/1994
WO95/03821	Prosaposin And Cytokine-Derived Peptides As Therapeutic Agents	2/9/1995
WO95/09175	Ring-Expanded Nucleosides And Nucleotides	4/6/1995
WO95/11992	Antiviral Compounds	5/4/1995
WO95/31198	Thiadiazoles And Their Use As Antipicornaviral Agents	11/23/1995
WO95/31438	Therapeutic Phenoxyalkylheterocycles	11/23/1995
WO95/31439	Therapeutic Phenoxyalkylpyridazines And Intermediates Therefor	11/23/1995
WO95/31452	Therapeutic Phenoxyalkylazoles And Phenoxyalkylazines	11/23/1995
WO95/34595	Antiviral Dendrimers	12/21/1995
WO95/35103	A Pharmaceutical Composition For The Prevention And/Or Treatment Of Viral Infections And Optionally Inflammations As Well As A Method For The Treatment Thereof	12/28/1995
WO96/05836	Methods Of Treating Cold Symptoms Using Pentoxifylline	2/29/1996
WO96/05854	Combination Preparation, Containing Cyclosporin A Or Fk506 Or Rapamycin And A Xanthine Derivative	2/29/1996
WO96/09822	Antipicornaviral Agents	4/4/1996
WO96/11211	Selective Inhibition Of Internally Initiated Rna Translation	4/18/1996
WO96/22689	Multiple Component Rna Catalysts And Uses Thereof	8/1/1996
WO96/40641	Sulfonamide Derivatives As Cell Adhesion Modulators	12/19/1996
WO97/08553	Targeting Of Proteins To The Cell Wall Of Gram-Positive Bacteria	3/6/1997
WO97/34566	Electrophilic Ketones For The Treatment Of Herpesvirus Infections	9/25/1997
WO97/41137	Use Of Anthocyanidin And Anthocyanidin Derivatives	11/6/1997
WO97/43305	Inhibitors Of Picornavirus 3c Proteases And Methods For Their Use And Preparation	11/20/1997

WO97/47270	Novel Anti-Viral Compounds	12/18/1997
WO98/03572	Antiviral Linear Polymers	1/29/1998
WO98/07745	Compositions And Methods For Treating Infections Using Analogues Of Indolicidin	2/26/1998
WO98/11778	Antimicrobial Treatment For Herpes Simplex Virus And Other Infectious Diseases	3/26/1998
WO98/22495	Antikinin Compounds And Uses Thereof	5/28/1998
WO98/31363	Anti-Viral Compounds	7/23/1998
WO98/31374	Method Of Treating Rhinoviral Infections	7/23/1998
WO98/32427	Therapeutic Treatment And Prevention Of Infections With A Bioactive Material Encapsulated Within A Biodegradable-Biocompatible Polymeric Matrix	7/30/1998
WO98/34601	Method For Inhibiting Intracellular Viral Replication	8/13/1998
WO98/42188	Antimicrobial Prevention And Treatment Of Human Immunedeficiency Virus And Other Infectious Diseases	10/1/1998
WO98/43950	Antipicornaviral Compouds, Compositions Containing Them, And Methods For Their Use	10/8/1998
WO98/49190	Substituted Oxadiazole Cysteine Protease Inhibitors	11/5/1998
WO98/55120	Anti-Viral Compounds	12/10/1998
WO99/30699	Modulators Of Cysteine Protease	6/24/1999
WO99/31122	Antipicornaviral Compounds And Methods For Their Use And Preparation	6/24/1999
WO99/54317	Cysteine Protease Inhibitors	10/28/1999
WO99/55663	Inhibitors Of Impdh Enzyme	11/4/1999
WO99/57135	Antipicornaviral Compounds, Their Preparation And Use	11/11/1999
WO99/59587	Anti-Viral Compounds	11/25/1999
WO99/61437	Novel 2-Alkyl Substituted Imidazole Compounds	12/2/1999

Table 2. US Patents and Published International Patent Applications

Publication Number	Title	Publication Date
WO02/69903	Nucleosides, Preparation Thereof And Use As Inhibitors Of Rna Viral Polymerases	9/12/2002
WO02/48116	Inhibitors Of Hepatitis C Virus Ns3 Protease	6/20/2002
WO02/48157	Imidazolidinones And Their Related Derivatives As Hepatitis C Virus Ns3 Protease Inhibitors	6/20/2002
WO02/61048	In Vitro System For Replication Of Rna-Dependent Rna Polymerase (Rdrp) Viruses	8/8/2002
WO03/02518	Novel 2,4-Difluorobenzamide Derivatives As Antiviral Agents	1/9/2003
WO02/79187	Methoxy-1,3,5-Triazine Derivatives As Antiviral Agents	10/10/2002
WO01/78648	6-Methylnicotinamide Derivatives As Antiviral Agents	10/25/2001
WO01/12214	MYCOPHENOLATE MOFETIL IN ASSOCIATION WITH PEG-IFN-.Alpha.	2/22/2001
WO02/100415	4'-Substituted Nucleosides	12/19/2002
WO02/18404	Nucleoside Derivatives	3/7/2002
WO02/94289	Antiviral Nucleoside Derivatives	11/28/2002
WO96/39500	Oligonucleotides Specific For Hepatitis C Virus	12/12/1996
WO03/00713	Nucleoside Compounds In Hcv	1/3/2003
WO01/60381	Nucleoside Analogs With Carboxamidine-Modified Bicyclic Base	8/23/2001
WO02/03997	Pyrido[2,3-D]Pyrimidine And Pyrimido[4,5-D]Pyrimidine Nucleosides	1/17/2002
WO97/26883	Modulation Of Th1/Th2 Cytokine Expression By Ribavirin3 And Ribavirin3 Analogs In Activated T-Lymphocytes	7/31/1997
WO03/26589	Methods And Compositions For Treating Hepatitis C Virus Using 4'-Modified Nucleosides	4/3/2003
WO03/26675	Methods And Compositions For Treating Flaviviruses And Pestiviruses Using 4'-Modified Nucleoside	4/3/2003
WO97/30067	Sugar-Modified Gapped Oligonucleotides	8/21/1997
WO01/47883	Fused-Ring Compounds And Use Thereof As Drugs	7/5/2001
WO03/00254	Fused Cyclic Compounds And Medicinal Use Thereof	1/3/2003
WO02/100354	Pyrrolo[2,3-D]Pyrimidine Nucleoside Analogs	12/19/2002
WO01/55111	Biaryl Compounds, Their Preparation And Their Use In Therapy	8/2/2001

WO01/16149	2-Azapurine Compounds And Their Use	3/8/2001
WO01/85770	Sentinel Virus Ii	11/15/2001
WO02/12263	Nucleic Acid Binding Compounds Containing Pyrazolo[3,4-D]Pyrimidine Analogues Of Purin-2,6-Diamine And Their Uses	2/14/2002
JP 2001-247550 A2	Condensed Ring Compound And Its Medicinal Use	9/11/2001
6210675	PT-NANB Hepatitis Polypeptides	4/3/2001
6451991	Sugar-Modified Gapped Oligonucleotides	9/17/2002
5830455	Method Of Treatment Using A Therapeutic Combination Of α-Interferon And Free Radical Scavengers	11/3/1998
5908621	Polyethylene Glycol Modified Interferon Therapy	6/1/1999
5990276	Synthetic Inhibitors Of Hepatitis C Virus NS3 Protease	11/23/1999
6172046	Combination Therapy For Eradicating Detectable HCV-RNA In Patients Having Chronic Hepatitis C Infection	1/9/2001
6177074	Polyethylene Glycol Modified Interferon Therapy	1/23/2001
6326137	Hepatitis C Virus Protease-Dependent Chimeric Pestivirus	12/4/2001
6434489	Compositions Of Hepatitis C Virus NS5B Polymerase And Methods For Crystallizing Same	8/13/2002
6461605	Continuous Low-Dose Cytokine Infusion Therapy	10/8/2002
6472373	Combination Therapy For Eradicating Detectable HCV-RNA In Antiviral Treatment Naive Patients Having Chronic Hepatitis C Infection	10/29/2002
6524570	Polyethylene Glycol Modified Interferon Therapy	2/25/2003
WO00/37097	Ribavirin-Interferon Alfa Induction Hcv Combination Therapy	6/29/2000
WO00/37110	Ribavirin-Pegylated Interferon Alfa Induction Hcv Combination Therapy	6/29/2000
WO00/62799	Hcv Combination Therapy, Containing Ribavirin In Association With Antioxidants	10/26/2000
WO01/58929	Azapeptides Useful In The Treatment Of Hepatitis C	8/16/2001
WO02/32414	Ribavirin-Pegylated Interferon Alfa Hcv Combination Therapy	4/25/2002
WO03/24461	Hcv Combination Therapy	3/27/2003
WO93/20835	Treatment Of Hepatitis With Gm-Csf	10/28/1993
WO96/36702	Soluble, Active Hepatitis C Virus Protease	11/21/1996
WO97/16204	Continuous Low-Dose Cytokine Infusion Therapy	5/9/1997
WO97/43310	Synthetic Inhibitors Of Hepatitis C Virus Ns3 Protease	11/20/1997
WO98/48840	Polyethylene Glycol-Interferon Alpha Conjugates For Therapy Of Infection	11/5/1998
WO99/15194	Combination Therapy For Eradicating Detectable Hcv-Rna In Patients Having Chronic Hepatitis C Infection	4/1/1999
WO99/59621	Combination Therapy Comprising Ribavirin And Interferon Alpha In Antiviral Treatment Naive Patients Having G Chronic Hepatitis C Infection	11/25/1999
WO02/100846	Compounds And Methods For The Treatment Or Prevention Of Flavivirus Infections	12/19/2002
WO02/100851	Compounds And Methods For The Treatment Or Prevention Of Flavivirus Infections	12/19/2002
5241053	Fused Proteins Comprising Glycoprotein Gd Of HSV-1 And LTB	8/31/1993
5556946	Interleukin-2/Viral Antigen Protein Chimers	9/17/1996
6087484	Enhancement Of Ribozyme Catalytic Activity By A 2'-O-Substituted Facilitator Oligonucleotide	7/11/2000
5830905	Compounds, Compositions And Methods For Treatment Of Hepatitis C	11/3/1998
6316492	Methods For Treating Or Preventing Viral Infections And Associated Diseases	11/13/2001
6440985	Methods For Treating Viral Infections	8/27/2002
WO00/10573	Compounds, Compositions And Methods For Treating Or Preventing Viral Infections And Associated Diseases	3/2/2000
WO00/13708	Methods For Treating Or Preventing Viral Infections And Associated Diseases	3/16/2000
WO00/18231	Methods For Treating Or Preventing Viral Infections And Associated Diseases	4/6/2000
WO99/51781	Hepatitis C Virus Ns5b Compositions And Methods Of Use Thereof	10/14/1999
6323180	Hepatitis C Inhibitor Tri-Peptides	11/27/2001
6143715	Hepatitis C Inhibitor Peptide Analogues	11/7/2000
6329379	Hepatitis C Inhibitor Tri-Peptides	12/11/2001
6329417	Hepatitis C Inhibitor Tri-Pepitides	12/11/2001

6410531	Hepatitis C Inhibitor Tri-Peptides	6/25/2002
6420380	Hepatitis C Inhibitor Tri-Peptides	7/16/2002
6448281	Viral Polymerase Inhibitors	9/10/2002
6479508	Viral Polymerase Inhibitors	11/12/2002
6534523	Hepatitis C Inhibitor Tri-Peptides	3/18/2003
WO00/09543	Hepatitis C Inhibitor Tri-Peptides	2/24/2000
WO00/09558	Hepatitis C Inhibitor Peptides	2/24/2000
WO00/59929	Macrocyclic Peptides Active Against The Hepatitis C Virus	10/12/2000
WO02/04425	Viral Polymerase Inhibitors	1/17/2002
WO02/70739	Hcv Polymerase Inhibitor Assay	9/12/2002
WO03/07945	Viral Polymerase Inhibitors	1/30/2003
WO03/10140	Viral Polymerase Inhibitors	2/6/2003
WO03/10141	Viral Polymerase Inhibitors	2/6/2003
WO99/07734	Hepatitis C Inhibitor Peptide Analogues	2/18/1999
WO01/16379	Hepatitis C Virus Replication Inhibitors	3/8/2001
WO02/07761	Inhibiting Hepatitis C Virus Processing And Replication	1/31/2002
WO02/57287	Nucleoside Derivatives As Inhibitors Of Rna-Dependent Rna Viral Polymerase	7/25/2002
WO02/57425	Nucleoside Derivatives As Inhibitors Of Rna-Dependent Rna Viral Polymerase	7/25/2002
WO02/70651	Viral Reporter Particles	9/12/2002
WO03/20222	Dioxolane And Oxathiolane Derivatives As Inhibitors Of Rna-Dependent Rna Viral Polymerase	3/13/2003
PCT/US2003/041493	Thiosemicarbazones as Anti-Virals and Immunopotentiators	01/10/2003

Table 3: US Patents and published international patent applications relating to inhalation technology for the delivery of antiviral compounds of the invention.

Publication Number	Title	Publication Date
5740794	Apparatus and methods for dispersing dry powder medicaments	4/21/1998
5775320	Method and device for delivering aerosolized medicaments	7/7/1998
5785049	Method and apparatus for dispersion of dry powder medicaments	7/28/1998
5814607	Pulmonary delivery of active fragments of parathyroid hormone	9/29/1998
5826633	Powder filling systems, apparatus and methods	10/27/1998
5458135	Method and device for delivering aerosolized medicaments	10/17/1995
5607915	Pulmonary delivery of active fragments of parathyroid hormone	3/4/1997
5654007	Methods and system for processing dispersible fine powders	8/5/1997
5922354	Methods and system for processing dispersible fine powders	7/13/1999
5928469	Process for storage of materials	7/27/1999
5976574	Processes for spray drying hydrophobic drugs in organic solvent suspensions	11/2/1999
5985248	Processes for spray drying solutions of hydrophobic drugs and compositions thereof	11/16/1999
5994314	Compositions and methods for nucleic acid delivery to the lung	11/30/1999
5997848	Methods and compositions for pulmonary delivery of insulin	12/7/1999
6001336	Processes for spray drying aqueous suspensions of hydrophobic drugs and compositions thereof	12/14/1999
6019968	Dispersible antibody compositions and methods for their preparation and use	2/1/2000
6051256	Dispersible macromolecule compositions and methods for their preparation and use	4/18/2000
6071428	Stable compositions	6/6/2000
6077543	Systems and processes for spray drying hydrophobic drugs with hydrophilic excipients	6/20/2000
6080721	Pulmonary delivery of active fragments of parathyroid hormone	6/27/2000
6089228	Apparatus and methods for dispersing dry powder medicaments	7/18/2000
6103270	Methods and system for processing dispersible fine powders	8/15/2000
6123936	Methods and compositions for the dry powder formulation of interferons	9/26/2000

6136346	Powdered pharmaceutical formulations having improved dispersibility	10/24/2000
6138668	Method and device for delivering aerosolized medicaments	10/31/2000
6165463	Dispersible antibody compositions and methods for their preparation and use	12/26/2000
6182712	Power filling apparatus and methods for their use	2/6/2001
6187344	Powdered pharmaceutical formulations having improved dispersibility	2/13/2001
6207135	Gaseous microparticles for ultrasonic diagnosis and process for their production	3/27/2001
6231851	Methods and compositions for the dry powder formulation of interferons	5/15/2001
6257233	Dry powder dispersing apparatus and methods for their use	7/10/2001
6258341	Stable glassy state powder formulations	7/10/2001
6267155	Powder filling systems, apparatus and methods	7/31/2001
6294204	Method of producing morphologically uniform microcapsules and microcapsules produced by this method	9/25/2001
6303582	Compositions and methods for nucleic acid delivery to the lung	10/16/2001
6309623	Stabilized preparations for use in metered dose inhalers	10/30/2001
6309671	Stable glassy state powder formulations	10/30/2001
6358530	Powdered pharmaceutical formulations having improved dispersibility	3/19/2002
6365190	Systems and processes for spray drying hydrophobic drugs with hydrophilic excipients	4/2/2002
6372258	Methods of spray-drying a drug and a hydrophobic amino acid	4/16/2002
6423344	Dispersible macromolecule compositions and methods for their preparation and use	7/23/2002
6426210	Storage of materials	7/30/2002
6433040	Stabilized bioactive preparations and methods of use	8/13/2002
6440337	Method and apparatus for the formation of particles	8/27/2002
RE37872	Storage of materials	10/8/2002
6479049	Methods and compositions for the dry powder formulation of interferons	11/12/2002
6503411	Stable compositions	1/7/2003
6509006	Devices compositions and methods for the pulmonary delivery of aerosolized medicaments	1/21/2003
6514496	Dispersible antibody compositions and methods for their preparation and use	2/4/2003
6518239	dry powder compositions having improved dispersivity	2/11/2003
6543448	apparatus and methods for dispersing dry powder medicaments	4/8/2003
6546929	dry powder dispersing apparatus and methods for their use	4/15/2003
WO 00/15262	dry powder active agent pulmonary delivery	3/23/2000
WO 93/00951	method and device for delivering aerosolized medicaments	1/21/1993
WO 94/07514	pulmonary delivery of active fragments of parathyroid hormone	4/14/1994
WO 95/24183	methods and compositions for pulmonary delivery of insulin	9/14/1995
WO 95/31479	methods and compositions for the dry powder formulation of interferons	11/23/1995
WO 96/09085	apparatus and methods for dispersing dry powder medicaments	3/28/1996
WO 96/32096	powdered pharmaceutical formulations having improved dispersibility	10/17/1996
WO 96/32116	compositions and methods for nucleic acid delivery to the lung	10/17/1996
WO 96/32149	pulmonary delivery of aerosolized medicaments	10/17/1996
WO 96/32152	pulmonary administration of dry powder alpha 1-antitrypsin	10/17/1996
WO 96/40068	methods and system for processing dispersible fine powders	12/19/1996
WO 97/41031	powder filling systems, apparatus and methods	11/6/1997
WO 97/41833	dispersible macromolecule compositions and methods for their preparation and use	11/13/1997
WO 98/16205	stable glassy state powder formulations	4/23/1998
WO 98/29096	aerosolized hydrophobic drug	7/9/1998
WO 98/29098	processes for spray drying aqueous suspensions of hydrophobic drugs with hydrophilic excipients and compositions prepared by such processes	7/9/1998
WO 98/29140	processes and compositions for spray drying hydrophobic drugs in organic solvent suspensions of hydrophilic excipients	7/9/1998
WO 98/29141	processes for spray drying solutions of hydrophobic drugs with hydrophilic excipients and compositions prepared by such processes	7/9/1998
WO 99/19215	powder filling apparatus and method	4/22/1999
WO 99/42124	liquid crystal forms of cyclosporin	8/26/1999

WO 99/47196	aerosolized active agent delivery	9/23/1999
WO 99/62495	dry powder dispersing apparatus and methods for their use	12/9/1999
WO 00/21594	flow resistance modulated aerosolized active agent delivery	4/20/2000
WO 00/61178	pulmonary administration of dry powder formulations for treating infertility	10/19/2000
WO 00/72904	apparatus and method for dispensing metered amount of aerosolized medication	12/7/2000
WO 01/00263	systems and methods for aerosolizing pharmaceutical formulations	1/4/2001
WO 01/00312	spray drying process for preparing dry powders	1/4/2001
WO 01/32144	dry powder compositions having improved dispersivity	5/10/2001
WO 01/43529	receptacles to facilitate the extraction of powders	6/21/2001
WO 01/43530	systems and methods for extracting powders from receptacles	6/21/2001
WO 01/43802	systems and methods for treating packaged powders	6/21/2001
WO 01/44764	systems and methods for non-destructive mass sensing	6/21/2001
WO 01/87393	systems, devices and methods for opening receptacles having a powder to be fluidized	11/22/2001
WO 01/93932	lockout mechanism for aerosol drug delivery devices	12/13/2001
WO 02/09669	apparatus and process to produce particles having a narrow size distribution and particles made thereby	2/7/2002
WO 02/11695	inhaleable spray dried 4-helix bundle protein powders having minimized aggregation	2/14/2002
WO 02/49619	induced phase transition method for the production of microparticles containing hydrophilic active agents	6/27/2002
WO 02/49620	induced phase transition method for the production of microparticles containing hydrophobic active agents	6/27/2002
WO 02/54868	pulmonary delivery of polyene antifungal agents	7/18/2002
WO 02/87542	novel methods and compositions for delivering macromolecules to or via the respiratory tract	11/7/2002
WO 02/100548	centrifuged rotating drum for treating cohesive powders	12/19/2002
WO 03/00326	powder aerosolization apparatus and method	1/3/2003
WO 03/00329	flow regulator for aerosol drug delivery device and methods	1/3/2003

TABLE 4: Forward and reverse primers for nucleic acid amplification of SARSV

Pair Number	Forward primer SEQ ID NO	Forward Primer Start	Forward Primer Stop	Forward Primer Tm	Forward Primer %GC	Reverse primer SEQ ID NO	Reverse Primer Start	Reverse Primer Stop	Reverse Primer Tm	Reverse Primer %GC	Primer Tm Diff	Product Length	Product Tm	Product %GC	Anneal Score	Optimum Anneal Temp
1	1021	12726	12746	51.3	47.6	3521	12996	12977	50.2	40	1	271	75	42.8	26	52.6
2	1022	12236	12256	51.2	42.9	3522	12993	12975	51.4	47.4	0.2	758	76.4	42.5	26	54
3	1023	12373	12391	50.8	47.4	3523	12993	12975	51.4	47.4	0.6	621	76.4	43	26	53.8
4	1024	12236	12256	51.2	42.9	3524	12996	12977	50.2	40	0.9	761	76.4	42.3	26	53.6
5	1025	12373	12391	50.8	47.4	3525	12996	12977	50.2	40	0.5	624	76.4	42.8	26	53.6
6	1026	12726	12746	51.3	47.6	3526	12993	12975	51.4	47.4	0.1	268	75.1	43.3	26	53.1
7	1027	2671	2692	52.1	40.9	3527	3185	3164	51	45.5	1.2	515	75.6	41.6	26	53.3
8	1028	28942	28961	50.2	45	3528	29298	29280	51.4	52.6	1.2	357	76.4	44.8	26	53.6
9	1029	19801	19819	53.2	52.6	3529	19922	19901	51.5	45.5	1.7	122	72.2	43.4	26	51.1
10	1030	19800	19817	50.4	50	3530	19921	19901	50.2	47.6	0.3	122	72.2	43.4	26	50.7
11	1031	9930	9948	51.5	52.6	3531	10605	10588	51.1	50	0.4	676	75.8	41.3	27	53.5
12	1032	9933	9952	50.9	45	3532	10605	10588	51.1	50	0.2	673	75.8	41.2	27	53.4
13	1033	9930	9949	52.2	50	3533	10605	10588	51.1	50	1.1	676	75.8	41.3	27	53.5

14	1034	9927	9945	50.8	52.6	3534	10605	10588	51.1	50	0.3	679	75.8	41.2	28	53.4
15	1035	3789	3806	50	50	3535	4445	4425	50.6	42.9	0.5	657	75.5	40.5	28	52.9
16	1036	3788	3805	50	50	3536	4444	4424	50.6	42.9	0.5	657	75.5	40.5	28	52.9
17	1037	3795	3813	52.1	52.6	3537	4445	4425	50.6	42.9	1.5	651	75.5	40.6	28	53.1
18	1038	3787	3804	50	50	3538	4445	4425	50.6	42.9	0.5	659	75.4	40.4	28	52.9
19	1039	19801	19819	53.2	52.6	3539	19921	19900	51.8	45.5	1.4	121	72.3	43.8	28	51.2
20	1040	24418	24436	50	47.4	3540	25182	25164	51.4	47.4	1.4	765	76.1	41.7	28	53.4
21	1041	9929	9949	53.8	47.6	3541	10449	10425	54.6	40	0.8	521	75.4	40.9	28	54
22	1042	2671	2692	52.1	40.9	3542	3186	3165	50.4	40.9	1.7	516	75.6	41.5	28	53.1
23	1043	3792	3810	52.9	52.6	3543	4446	4425	51.8	45.5	1.1	655	75.5	40.6	28	53.5
24	1044	9933	9952	50.9	45	3544	10449	10431	50.9	47.4	0.1	517	75.3	40.8	28	53.1
25	1045	3792	3810	52.9	52.6	3545	4445	4424	51.3	40.9	1.6	654	75.5	40.5	28	53.3
26	1046	25782	25806	53.5	40	3546	26184	26164	52.4	42.9	1.1	403	74.7	40.2	28	53.1
27	1047	9927	9945	50.8	52.6	3547	10449	10431	50.9	47.4	0.1	523	75.4	40.9	28	53.1
28	1048	9927	9945	50.8	52.6	3548	10449	10428	51.9	40.9	1.1	523	75.4	40.9	28	53.1
29	1049	3789	3806	50	50	3549	4444	4424	50.6	42.9	0.5	656	75.5	40.5	28	53
30	1050	3795	3813	52.1	52.6	3550	4444	4424	50.6	42.9	1.5	650	75.5	40.6	28	53.1
31	1051	9933	9952	50.9	45	3551	10449	10428	51.9	40.9	1.1	517	75.3	40.8	28	53.1
32	1052	9930	9948	51.5	52.6	3552	10449	10431	50.9	47.4	0.5	520	75.4	41	28	53.2
33	1053	9930	9948	51.5	52.6	3553	10449	10428	51.9	40.9	0.4	520	75.4	41	28	53.3
34	1054	9929	9948	53.2	50	3554	10449	10425	54.6	40	1.4	521	75.4	40.9	28	53.8
35	1055	9931	9952	53	45.5	3555	10449	10425	54.6	40	1.6	519	75.3	40.8	28	53.7
36	1056	3791	3808	50	50	3556	4445	4425	50.6	42.9	0.5	655	75.5	40.5	28	52.9
37	1057	3791	3808	50	50	3557	4444	4424	50.6	42.9	0.5	654	75.5	40.5	28	53
38	1058	9930	9949	52.2	50	3558	10449	10431	50.9	47.4	1.2	520	75.4	41	28	53.2
39	1059	9930	9949	52.2	50	3559	10449	10428	51.9	40.9	0.3	520	75.4	41	28	53.5
40	1060	3788	3805	50	50	3560	4445	4425	50.6	42.9	0.5	658	75.5	40.4	28	52.9
41	1061	19800	19817	50.4	50	3561	19921	19900	51.8	45.5	1.4	122	72.2	43.4	28	50.8
42	1062	3787	3804	50	50	3562	4444	4424	50.6	42.9	0.5	658	75.5	40.4	28	52.9
43	1063	25782	25806	53.5	40	3563	26183	26163	51.7	42.9	1.7	402	74.7	40.3	28	52.9
44	1064	25782	25806	53.5	40	3564	26183	26160	54.5	41.7	1	402	74.7	40.3	28	53.5
45	1065	25782	25806	53.5	40	3565	26183	26159	54.9	40	1.5	402	74.7	40.3	28	53.5
46	1066	2429	2447	50.2	47.4	3566	3187	3166	50.3	45.5	0.1	759	76.6	43	29	53.8
47	1067	2427	2445	52.1	52.6	3567	3185	3164	51	45.5	1.1	759	76.7	43.1	29	54.1
48	1068	2429	2447	50.2	47.4	3568	3185	3164	51	45.5	0.7	757	76.6	42.9	29	53.8
49	1069	19800	19817	50.4	50	3569	19923	19904	50.1	50	0.3	124	72.3	43.5	29	50.8
50	1070	2427	2445	52.1	52.6	3570	3187	3166	50.3	45.5	1.8	761	76.7	43.1	29	53.9
51	1071	29183	29204	50.4	40.9	3571	29412	29393	50.3	45	0	230	75.3	44.8	29	52.9
52	1072	16367	16386	51.4	50	3572	16780	16760	51.4	42.9	0.1	414	75	40.8	30	53
53	1073	11543	11562	50.4	40	3573	12254	12236	50.5	47.4	0.1	712	76.2	42	30	53.6
54	1074	12976	12995	51.1	45	3574	13547	13528	50.2	45	0.9	572	77.4	45.5	30	54.3
55	1075	12040	12057	50.6	50	3575	12254	12236	50.5	47.4	0.1	215	75.5	45.6	30	53.1
56	1076	12976	12996	51.8	42.9	3576	13544	13525	52.6	55	0.8	569	77.5	45.7	30	54.8
57	1077	10141	10160	51	45	3577	10605	10588	51.1	50	0.1	465	74.9	40.2	30	52.8
58	1078	12235	12253	50.1	52.6	3578	12996	12977	50.2	40	0.1	762	76.4	42.4	30	53.6
59	1079	19795	19814	50.4	45	3579	19921	19901	50.2	47.6	0.3	127	72.3	43.3	30	50.8
60	1080	12235	12253	50.1	52.6	3580	12993	12975	51.4	47.4	1.3	759	76.5	42.6	30	53.7
61	1081	12976	12994	50.3	47.4	3581	13547	13528	50.2	45	0.1	572	77.4	45.5	30	54.3
62	1082	12975	12994	52.1	45	3582	13544	13525	52.6	55	0.5	570	77.4	45.6	30	54.9
63	1083	12977	12996	50.2	40	3583	13547	13528	50.2	45	0	571	77.3	45.4	30	54.3
64	1084	11541	11561	50.9	42.9	3584	12254	12236	50.5	47.4	0.3	714	76.2	42	30	53.6
65	1085	28394	28411	50.3	50	3585	28672	28654	50.6	52.6	0.3	279	78.6	51.6	30	55.2

66	1086	9930	9948	51.5	52.6	3586	10455	10434	51.1	40.9	0.3	526	75.3	40.7	30	53.1
67	1087	8220	8238	51.5	47.4	3587	8929	8911	53.4	52.6	1.9	710	75.4	40	30	53.3
68	1088	9930	9949	52.2	50	3588	10455	10435	50.5	42.9	1.7	526	75.3	40.7	30	52.9
69	1089	12236	12256	51.2	42.9	3589	12412	12392	50	42.9	1.2	177	73	41.2	30	51.2
70	1090	9930	9949	52.2	50	3590	10455	10434	51.1	40.9	1.1	526	75.3	40.7	30	53.1
71	1091	9933	9952	50.9	45	3591	10455	10435	50.5	42.9	0.4	523	75.2	40.5	30	52.9
72	1092	12726	12746	51.3	47.6	3592	13314	13297	51	50	0.3	589	76.6	43.6	30	54
73	1093	9933	9952	50.9	45	3593	10455	10434	51.1	40.9	0.3	523	75.2	40.5	30	53
74	1094	16909	16928	50.8	45	3594	17501	17481	51.2	42.9	0.4	593	75.9	41.8	30	53.5
75	1095	12975	12993	51.4	47.4	3595	13544	13525	52.6	55	1.2	570	77.4	45.6	30	54.7
76	1096	2671	2692	52.1	40.9	3596	3187	3166	50.3	45.5	1.8	517	75.6	41.6	30	53.1
77	1097	19800	19818	52.1	52.6	3597	19921	19900	51.8	45.5	0.3	122	72.2	43.4	30	51.2
78	1098	12975	12993	51.4	47.4	3598	13547	13528	50.2	45	1.2	573	77.3	45.4	30	54.3
79	1099	9930	9948	51.5	52.6	3599	10455	10435	50.5	42.9	1	526	75.3	40.7	30	52.9
80	1100	12976	12995	51.1	45	3600	13544	13525	52.6	55	1.5	569	77.5	45.7	30	54.6
81	1101	24635	24653	50.5	52.6	3601	25182	25164	51.4	47.4	0.9	548	75.1	40.1	30	52.8
82	1102	24633	24651	50.1	52.6	3602	25182	25164	51.4	47.4	1.3	550	75.2	40.2	30	52.7
83	1103	24630	24648	50.8	52.6	3603	25182	25164	51.4	47.4	0.6	553	75.2	40.3	30	53
84	1104	28394	28412	51.1	47.4	3604	28672	28654	50.6	52.6	0.5	279	78.6	51.6	30	55.3
85	1105	28395	28413	50.2	42.1	3605	28672	28654	50.6	52.6	0.4	278	78.6	51.4	30	55.2
86	1106	28396	28415	51.2	45	3606	28672	28654	50.6	52.6	0.6	277	78.6	51.6	30	55.3
87	1107	26421	26441	51.5	42.9	3607	26587	26568	52.7	45	1.2	167	72.3	40.1	30	51.2
88	1108	26421	26441	51.5	42.9	3608	26589	26571	51.7	47.4	0.2	169	72.4	40.2	30	51.2
89	1109	26421	26441	51.5	42.9	3609	26589	26572	51	50	0.5	169	72.4	40.2	30	51.1
90	1110	26421	26441	51.5	42.9	3610	26590	26573	51.7	50	0.3	170	72.3	40	30	51.2
91	1111	26040	26061	56.4	54.5	3611	26589	26568	55.2	45.5	1.2	550	75.1	40	30	54.2
92	1112	26039	26057	52.6	52.6	3612	26183	26160	54.5	41.7	1.9	145	71.9	40.7	30	51.2
93	1113	26039	26057	52.6	52.6	3613	26182	26161	51.2	40.9	1.4	144	71.7	40.3	30	50.7
94	1114	26039	26057	52.6	52.6	3614	26183	26163	51.7	42.9	0.9	145	71.9	40.7	30	51
95	1115	8867	8887	52.3	47.6	3615	9253	9235	51.6	47.4	0.7	387	75.1	41.3	30	53.2
96	1116	10247	10267	50.5	47.6	3616	10605	10588	51.1	50	0.6	359	74.6	40.4	30	52.4
97	1117	11540	11557	50.4	50	3617	12254	12236	50.5	47.4	0.1	715	76.2	42.1	30	53.6
98	1118	11541	11560	50.1	45	3618	12254	12236	50.5	47.4	0.4	714	76.2	42	30	53.5
99	1119	8221	8240	52.4	50	3619	8929	8911	53.4	52.6	1	709	75.4	40.1	30	53.6
100	1120	13039	13057	51.1	52.6	3620	13177	13156	50.4	40.9	0.7	139	73.9	46	31	52
101	1121	19801	19819	53.2	52.6	3621	19917	19895	52.5	43.5	0.8	117	72	43.6	31	51.2
102	1122	19709	19730	51.3	40.9	3622	19921	19900	51.8	45.5	0.5	213	73.9	41.8	31	52.2
103	1123	16366	16386	54.4	52.4	3623	16774	16751	53.6	41.7	0.8	409	75.1	41.1	31	53.8
104	1124	3	21	53.4	52.6	3624	256	235	52.6	45.5	0.8	254	76.1	46.1	31	54.2
105	1125	4	22	52.3	52.6	3625	314	296	50.6	47.4	1.7	311	76.8	46.6	31	54.1
106	1126	13039	13058	51.8	50	3626	13177	13156	50.4	40.9	1.5	139	73.9	46	31	52
107	1127	19800	19817	50.4	50	3627	19916	19895	50.2	40.9	0.2	117	71.7	42.7	31	50.3
108	1128	4645	4665	50.2	42.9	3628	5306	5289	50.8	50	0.5	662	75.6	40.8	31	53.1
109	1129	13039	13057	51.1	52.6	3629	13747	13726	50.8	40.9	0.4	709	76.6	43.2	31	54
110	1130	13039	13058	51.8	50	3630	13747	13726	50.8	40.9	1.1	709	76.6	43.2	31	54
111	1131	3	21	53.4	52.6	3631	253	233	51.8	47.6	1.6	251	76.2	46.2	31	54
112	1132	27365	27385	53.2	47.6	3632	27464	27444	53	42.9	0.2	100	70.8	43	31	50.6
113	1133	24418	24436	50	47.4	3633	24527	24508	50.5	45	0.5	110	71.3	42.7	31	50
114	1134	26708	26727	50	45	3634	27463	27446	50	44.4	0	756	75.9	41.1	31	53.2
115	1135	24179	24200	53.3	40.9	3635	24936	24919	51.8	50	1.5	758	75.8	41	31	53.7
116	1136	26708	26727	50	45	3636	27462	27444	50.1	42.1	0.1	755	75.9	41.2	31	53.2

117	1137	26708	26731	54.2	41.7	3637	27465	27446	54.6	50	0.4	758	75.9	41.3	31	54.5
118	1138	27365	27384	52.6	50	3638	27464	27446	51.7	47.4	0.9	100	70.8	43	31	50.2
119	1139	27365	27384	52.6	50	3639	27464	27445	52.4	45	0.2	100	70.8	43	31	50.4
120	1140	27365	27384	52.6	50	3640	27464	27444	53	42.9	0.4	100	70.8	43	31	50.4
121	1141	27367	27385	51.4	52.6	3641	27571	27552	50.1	40	1.3	205	74.6	43.9	31	52.4
122	1142	27367	27385	51.4	52.6	3642	27567	27547	51.1	42.9	0.2	201	74.7	44.3	31	52.7
123	1143	2427	2445	52.1	52.6	3643	3186	3165	50.4	40.9	1.7	760	76.7	43	31	53.9
124	1144	8867	8887	52.3	47.6	3644	9256	9237	50.8	45	1.5	390	75.1	41.3	31	52.9
125	1145	9934	9953	50.7	50	3645	10605	10588	51.1	50	0.4	672	75.8	41.2	31	53.4
126	1146	2429	2447	50.2	47.4	3646	3186	3165	50.4	40.9	0.2	758	76.6	42.9	31	53.8
127	1147	27365	27385	53.2	47.6	3647	27464	27445	52.4	45	0.8	100	70.8	43	31	50.4
128	1148	19994	20011	50.4	50	3648	20615	20597	50.6	47.4	0.2	622	75.2	40	31	52.9
129	1149	9922	9941	51.3	50	3649	10605	10588	51.1	50	0.2	684	75.8	41.2	32	53.5
130	1150	12962	12980	50.7	47.4	3650	13544	13525	52.6	55	1.8	583	77.5	45.6	32	54.5
131	1151	12965	12988	54	41.7	3651	13544	13525	52.6	55	1.5	580	77.4	45.5	32	55
132	1152	13176	13197	52.7	45.5	3652	13544	13525	52.6	55	0.1	369	77.1	46.3	32	54.8
133	1153	28867	28886	53.2	50	3653	29298	29280	51.4	52.6	1.7	432	76.8	45.1	32	54.3
134	1154	24418	24439	52.9	45.5	3654	25182	25164	51.4	47.4	1.5	765	76.1	41.7	32	53.8
135	1155	24420	24440	50.8	42.9	3655	25182	25164	51.4	47.4	0.6	763	76.1	41.5	32	53.6
136	1156	8867	8887	52.3	47.6	3656	9107	9086	51.6	45.5	0.7	241	74.1	41.5	32	52.5
137	1157	1402	1422	50.2	42.9	3657	2103	2083	50.6	42.9	0.4	702	76.7	43.3	32	53.8
138	1158	25782	25805	52.1	41.7	3658	26183	26163	51.7	42.9	0.4	402	74.7	40.3	32	52.9
139	1159	25781	25805	53.5	40	3659	26183	26160	54.5	41.7	1	403	74.7	40.2	32	53.4
140	1160	25781	25805	53.5	40	3660	26183	26159	54.9	40	1.5	403	74.7	40.2	32	53.4
141	1161	2671	2692	52.1	40.9	3661	3052	3033	50.3	50	1.8	382	74.8	40.6	32	52.5
142	1162	12726	12746	51.3	47.6	3662	13177	13156	50.4	40.9	0.9	452	76.4	43.8	32	53.7
143	1163	16909	16928	50.8	45	3663	17111	17090	51.1	40.9	0.3	203	75	44.8	32	52.8
144	1164	12234	12252	50.6	47.4	3664	12993	12975	51.4	47.4	0.8	760	76.4	42.5	32	53.8
145	1165	26039	26057	52.6	52.6	3665	26828	26810	52.9	52.6	0.2	790	76.4	42.4	32	54.4
146	1166	26039	26057	52.6	52.6	3666	26694	26677	51.4	50	1.2	656	75.7	41	32	53.5
147	1167	26039	26057	52.6	52.6	3667	26692	26674	51.9	52.6	0.7	654	75.7	41	32	53.6
148	1168	26039	26057	52.6	52.6	3668	26691	26673	51.3	47.4	1.3	653	75.6	40.9	32	53.4
149	1169	26039	26057	52.6	52.6	3669	26687	26669	51.3	47.4	1.3	649	75.6	40.8	32	53.4
150	1170	26039	26057	52.6	52.6	3670	26684	26666	53.4	52.6	0.8	646	75.6	40.9	32	53.8
151	1171	26039	26057	52.6	52.6	3671	26683	26665	52.7	52.6	0.1	645	75.6	40.9	32	53.8
152	1172	9934	9953	50.7	50	3672	10449	10431	50.9	47.4	0.2	516	75.4	40.9	32	53.1
153	1173	9927	9945	50.8	52.6	3673	10455	10434	51.1	40.9	0.3	529	75.3	40.6	32	53
154	1174	7728	7746	51.7	52.6	3674	8188	8169	50.5	45	1.2	461	75.6	41.9	32	53.2
155	1175	18550	18571	50.4	40.9	3675	19216	19195	50.2	40.9	0.2	667	75.7	41.1	32	53.2
156	1176	19801	19819	53.2	52.6	3676	19921	19899	52.4	43.5	0.8	121	72.3	43.8	32	51.4
157	1177	19709	19730	51.3	40.9	3677	19923	19904	50.1	50	1.2	215	73.9	41.9	32	51.9
158	1178	4639	4659	51.1	47.6	3678	5306	5289	50.8	50	0.3	668	75.6	40.9	32	53.3
159	1179	19794	19813	50	50	3679	19921	19901	50.2	47.6	0.2	128	72.6	43.8	32	50.9
160	1180	12965	12985	51.2	42.9	3680	13544	13525	52.6	55	1.4	580	77.4	45.5	32	54.6
161	1181	9932	9953	53	45.5	3681	10449	10425	54.6	40	1.6	518	75.3	40.7	32	53.7
162	1182	19795	19814	50.4	45	3682	19921	19900	51.8	45.5	1.4	127	72.3	43.3	32	50.9
163	1183	27366	27384	52.2	52.6	3683	27468	27451	51.1	50	1	103	71.3	43.7	32	50.3
164	1184	27366	27384	52.2	52.6	3684	27467	27450	52.1	50	0.1	102	71.4	44.1	32	50.7
165	1185	27366	27384	52.2	52.6	3685	27466	27449	51	50	1.2	101	71.5	44.6	32	50.4
166	1186	25782	25805	52.1	41.7	3686	26183	26164	51	45	1.1	402	74.7	40.3	32	52.7
167	1187	9934	9953	50.7	50	3687	10449	10428	51.9	40.9	1.2	516	75.4	40.9	32	53.1
168	1188	9925	9945	53.4	52.4	3688	10449	10425	54.6	40	1.2	525	75.4	41	32	53.9

169	1189	19800	19817	50.4	50	3689	19922	19902	50	42.9	0.4	123	72.1	43.1	32	50.6
170	1190	8867	8887	52.3	47.6	3690	9310	9291	51.2	45	1.2	444	75.4	41.4	32	53.2
171	1191	27367	27385	51.4	52.6	3691	27468	27451	51.1	50	0.3	102	71.4	44.1	32	50.4
172	1192	27367	27385	51.4	52.6	3692	27467	27450	52.1	50	0.7	101	71.5	44.6	32	50.6
173	1193	2671	2692	52.1	40.9	3693	3082	3058	52.3	40	0.2	412	74.9	40.5	32	53.2
174	1194	9927	9945	50.8	52.6	3694	10608	10589	51	50	0.2	682	75.8	41.2	32	53.4
175	1195	19800	19817	50.4	50	3695	19920	19899	50.2	40.9	0.3	121	71.9	43	32	50.5
176	1196	13177	13197	50.3	42.9	3696	13547	13528	50.2	45	0.1	371	76.9	45.8	32	54
177	1197	28179	28200	50.8	40.9	3697	28672	28654	50.6	52.6	0.3	494	79.8	51.8	32	56.1
178	1198	27367	27385	51.4	52.6	3698	27466	27449	51	50	0.4	100	71.6	45	32	50.5
179	1199	27366	27385	52.8	50	3699	27465	27446	54.6	50	1.7	100	71.2	44	32	50.8
180	1200	19800	19818	52.1	52.6	3700	19921	19901	50.2	47.6	2	122	72.2	43.4	32	50.7
181	1201	9927	9945	50.8	52.6	3701	10455	10435	50.5	42.9	0.3	529	75.3	40.6	32	52.9
182	1202	28868	28887	50.7	45	3702	29298	29280	51.4	52.6	0.7	431	76.8	45	32	54.1
183	1203	28867	28887	53.7	47.6	3703	29306	29288	53.5	52.6	0.3	440	76.9	45.2	32	55
184	1204	28867	28887	53.7	47.6	3704	29301	29282	55.3	55	1.5	435	76.9	45.3	32	55.1
185	1205	28868	28888	51.4	42.9	3705	29298	29280	51.4	52.6	0	431	76.8	45	32	54.3
186	1206	28867	28888	54.3	45.5	3706	29306	29288	53.5	52.6	0.8	440	76.9	45.2	32	55
187	1207	28867	28888	54.3	45.5	3707	29301	29282	55.3	55	1	435	76.9	45.3	32	55.2
188	1208	28870	28889	50.1	40	3708	29298	29280	51.4	52.6	1.3	429	76.8	45	32	53.9
189	1209	28868	28889	52	40.9	3709	29306	29288	53.5	52.6	1.5	439	76.9	45.1	32	54.5
190	1210	28867	28889	54.8	43.5	3710	29301	29282	55.3	55	0.5	435	76.9	45.3	32	55.4
191	1211	28867	28890	55.2	41.7	3711	29306	29288	53.5	52.6	1.7	440	76.9	45.2	32	55
192	1212	28867	28890	55.2	41.7	3712	29301	29282	55.3	55	0.1	435	76.9	45.3	32	55.5
193	1213	28867	28890	55.2	41.7	3713	29299	29280	53.9	55	1.3	433	76.9	45.3	32	55.1
194	1214	12234	12252	50.6	47.4	3714	12996	12977	50.2	40	0.3	763	76.4	42.3	32	53.6
195	1215	28968	28988	50.9	47.6	3715	29298	29280	51.4	52.6	0.6	331	76.2	44.7	32	53.7
196	1216	28968	28989	51.5	45.5	3716	29298	29280	51.4	52.6	0.1	331	76.2	44.7	32	53.9
197	1217	13230	13251	52.4	45.5	3717	13544	13525	52.6	55	0.1	315	77.2	47.3	32	54.8
198	1218	29186	29205	50.1	40	3718	29298	29280	51.4	52.6	1.3	113	72.8	46	32	51.1
199	1219	29195	29213	51.9	52.6	3719	29306	29288	53.5	52.6	1.6	112	73.6	48.2	32	52.2
200	1220	29195	29213	51.9	52.6	3720	29298	29280	51.4	52.6	0.5	104	73.1	48.1	32	51.7
201	1221	29196	29214	51.1	52.6	3721	29298	29280	51.4	52.6	0.3	103	73.3	48.5	32	51.7
202	1222	29195	29214	52.6	50	3722	29306	29288	53.5	52.6	0.9	112	73.6	48.2	32	52.4
203	1223	29196	29215	51.8	50	3723	29306	29288	53.5	52.6	1.6	111	73.8	48.6	32	52.3
204	1224	29196	29215	51.8	50	3724	29298	29280	51.4	52.6	0.4	103	73.3	48.5	32	51.8
205	1225	29197	29216	50	45	3725	29298	29280	51.4	52.6	1.4	102	73	48	32	51.2
206	1226	29196	29216	52.5	47.6	3726	29306	29288	53.5	52.6	1	111	73.8	48.6	32	52.5
207	1227	29195	29216	53.8	45.5	3727	29301	29282	55.3	55	1.5	107	73.5	48.6	32	52.7
208	1228	29254	29273	53.1	50	3728	29358	29339	52.8	50	0.2	105	73.4	48.6	32	52.3
209	1229	29259	29278	52.6	50	3729	29358	29339	52.8	50	0.2	100	72.4	47	32	51.6
210	1230	1402	1422	50.2	42.9	3730	1773	1755	51.7	52.6	1.5	372	75.8	43.3	33	53.2
211	1231	12726	12746	51.3	47.6	3731	13326	13306	50.7	42.9	0.6	601	76.7	43.6	33	54
212	1232	4	22	52.3	52.6	3732	269	251	51.1	52.6	1.2	266	76.5	46.6	33	54
213	1233	19800	19817	50.4	50	3733	19923	19903	50.9	47.6	0.4	124	72.3	43.5	33	50.9
214	1234	2371	2389	50.3	47.4	3734	3082	3058	52.3	40	2	712	76.7	43.3	33	53.9
215	1235	3	21	53.4	52.6	3735	270	251	52.9	50	0.5	268	76.4	46.3	33	54.4
216	1236	9930	9949	52.2	50	3736	10183	10166	50.9	50	1.3	254	75.3	44.1	33	53.1
217	1237	19795	19814	50.4	45	3737	19923	19904	50.1	50	0.3	129	72.5	43.4	33	50.9
218	1238	8867	8887	52.3	47.6	3738	9365	9347	53	52.6	0.7	499	75.8	42.1	33	53.9
219	1239	2371	2389	50.3	47.4	3739	3055	3036	50.6	50	0.3	685	76.7	43.4	33	53.9

220	1240	19709	19730	51.3	40.9	3740	19921	19901	50.2	47.6	1.1	213	73.9	41.8	33	51.9
221	1241	9930	9949	52.2	50	3741	10183	10165	51.7	47.4	0.5	254	75.3	44.1	33	53.3
222	1242	2371	2389	50.3	47.4	3742	2747	2727	50	42.9	0.3	377	76.9	45.9	33	54
223	1243	24921	24938	50.4	50	3743	25182	25164	51.4	47.4	1	262	74.2	41.2	33	52.2
224	1244	18077	18099	54.4	47.8	3744	18443	18424	55.9	55	1.5	367	75.8	43.3	33	54.5
225	1245	25772	25793	52.4	40.9	3745	26183	26164	51	45	1.3	412	74.8	40.3	33	52.8
226	1246	25769	25786	50.3	50	3746	26183	26164	51	45	0.8	415	74.9	40.5	33	52.6
227	1247	25348	25366	51.2	47.4	3747	25548	25531	51.1	50	0.1	201	74.3	43.3	33	52.4
228	1248	12726	12746	51.3	47.6	3748	13323	13304	51.1	45	0.2	598	76.7	43.6	33	54.1
229	1249	8372	8390	50.7	47.4	3749	8928	8911	51.9	50	1.2	557	75.1	40	33	52.9
230	1250	2671	2692	52.1	40.9	3750	3189	3168	51	45.5	1.2	519	75.7	41.6	33	53.4
231	1251	25348	25365	50.4	50	3751	25548	25531	51.1	50	0.7	201	74.3	43.3	33	52.2
232	1252	19801	19819	53.2	52.6	3752	19923	19902	51.5	45.5	1.7	123	72.4	43.9	33	51.3
233	1253	27442	27461	51.5	40	3753	27546	27527	51.3	50	0.2	105	71.8	44.8	33	50.8
234	1254	8867	8887	52.3	47.6	3754	9312	9293	50.6	45	1.8	446	75.4	41.5	33	53
235	1255	2671	2692	52.1	40.9	3755	3056	3038	50.8	52.6	1.3	386	74.8	40.7	33	52.7
236	1256	13231	13251	50.1	42.9	3756	13547	13528	50.2	45	0.2	317	76.9	46.7	33	54
237	1257	9055	9079	52.8	40	3757	9310	9291	51.2	45	1.7	256	74.4	41.8	33	52.5
238	1258	28821	28838	50.3	50	3758	29298	29280	51.4	52.6	1.1	478	77	45.2	33	54.1
239	1259	9055	9079	52.8	40	3759	9253	9235	51.6	47.4	1.2	199	73.6	41.7	33	52.1
240	1260	23840	23863	55.2	45.8	3760	24050	24031	56.5	55	1.4	211	75	44.5	33	54.1
241	1261	18074	18093	50.3	45	3761	18233	18214	52	50	1.7	160	73.9	44.4	33	51.9
242	1262	27366	27384	52.2	52.6	3762	27674	27654	51.9	42.9	0.3	309	74.3	40.5	33	52.7
243	1263	28967	28989	53.7	47.8	3763	29301	29282	55.3	55	1.5	335	76.4	45.1	33	54.7
244	1264	27366	27384	52.2	52.6	3764	27674	27653	52.5	40.9	0.3	309	74.3	40.5	33	52.8
245	1265	28966	28988	55.3	52.2	3765	29301	29282	55.3	55	0.1	336	76.4	45.2	33	55.2
246	1266	18074	18094	51.1	42.9	3766	18233	18214	52	50	1	160	73.9	44.4	33	52.1
247	1267	28965	28984	52.9	55	3767	29298	29280	51.4	52.6	1.5	334	76.4	45.2	33	54
248	1268	18081	18099	51.2	52.6	3768	18233	18215	51.3	52.6	0.1	153	74	45.1	33	52.2
249	1269	18081	18099	51.2	52.6	3769	18233	18214	52	50	0.8	153	74	45.1	33	52.2
250	1270	18081	18099	51.2	52.6	3770	18231	18210	52.2	45.5	1	151	73.6	44.4	33	52
251	1271	24480	24500	53.2	47.6	3771	24815	24791	54.5	40	1.3	336	75.6	43.2	33	54
252	1272	24481	24503	52.7	43.5	3772	24815	24791	54.5	40	1.8	335	75.5	43	33	53.8
253	1273	27367	27385	51.4	52.6	3773	27675	27656	50	40	1.4	309	74.3	40.5	33	52.1
254	1274	27367	27385	51.4	52.6	3774	27674	27654	51.9	42.9	0.5	308	74.4	40.6	33	52.6
255	1275	27367	27385	51.4	52.6	3775	27674	27653	52.5	40.9	1.1	308	74.4	40.6	33	52.6
256	1276	18081	18099	51.2	52.6	3776	18223	18206	51.8	50	0.6	143	73.2	44.1	33	51.7
257	1277	18080	18099	53	50	3777	18220	18202	54.8	52.6	1.9	141	73.1	44	33	52.2
258	1278	9933	9952	50.9	45	3778	10670	10649	51.3	40.9	0.5	738	75.7	40.8	33	53.4
259	1279	27665	27686	51.4	40.9	3779	28208	28190	51.7	52.6	0.4	544	75.1	40.1	33	53.1
260	1280	27665	27685	50.7	42.9	3780	28208	28190	51.7	52.6	1.1	544	75.1	40.1	33	52.9
261	1281	27442	27461	51.5	40	3781	27541	27522	50.1	45	1.4	100	71.2	44	33	50
262	1282	28821	28840	51.8	45	3782	29298	29280	51.4	52.6	0.4	478	77	45.2	33	54.4
263	1283	28821	28839	51.1	47.4	3783	29298	29280	51.4	52.6	0.3	478	77	45.2	33	54.3
264	1284	8868	8889	50.4	40.9	3784	9252	9235	50.1	50	0.3	385	75.1	41.3	34	52.7
265	1285	19800	19818	52.1	52.6	3785	19920	19899	50.2	40.9	2	121	71.9	43	34	50.5
266	1286	9055	9079	52.8	40	3786	9313	9293	52.1	47.6	0.7	259	74.6	42.1	34	52.9
267	1287	10142	10163	51.3	40.9	3787	10605	10588	51.1	50	0.2	464	74.9	40.1	34	52.8
268	1288	12726	12746	51.3	47.6	3788	13312	13294	51	52.6	0.3	587	76.6	43.6	34	54
269	1289	9055	9079	52.8	40	3789	9257	9237	52.2	42.9	0.7	203	73.5	41.4	34	52.2
270	1290	7876	7895	51.5	45	3790	8188	8169	50.5	45	1.1	313	75	42.2	34	52.8

271	1291	23843	23863	50.3	42.9	3791	24527	24507	51	42.9	0.7	685	76	41.8	34	53.4
272	1292	10247	10267	50.5	47.6	3792	10608	10589	51	50	0.5	362	74.6	40.3	34	52.4
273	1293	24179	24199	52.7	42.9	3793	24815	24791	54.5	40	1.8	637	75.8	41.3	34	53.9
274	1294	12236	12256	51.2	42.9	3794	12998	12979	50.1	45	1.1	763	76.4	42.5	34	53.6
275	1295	7869	7889	52.5	47.6	3795	8189	8169	52	47.6	0.5	321	75.3	42.7	34	53.4
276	1296	1402	1422	50.2	42.9	3796	2152	2133	50.7	45	0.5	751	76.7	43.1	34	53.8
277	1297	12233	12251	51.1	52.6	3797	12993	12975	51.4	47.4	0.2	761	76.5	42.6	34	54
278	1298	3033	3053	51.7	47.6	3798	3650	3631	53.1	50	1.4	618	76.4	42.9	34	54.1
279	1299	12233	12251	51.1	52.6	3799	12996	12977	50.2	40	0.9	764	76.4	42.4	34	53.7
280	1300	24483	24503	51	42.9	3800	24938	24921	50.4	50	0.6	456	75.6	41.9	34	53.1
281	1301	11541	11561	50.9	42.9	3801	12253	12235	50.1	52.6	0.8	713	76.2	42.1	34	53.5
282	1302	24622	24643	57.1	54.5	3802	25400	25379	56	50	1.1	779	75.7	40.7	34	54.9
283	1303	24622	24643	57.1	54.5	3803	25400	25378	56.4	47.8	0.6	779	75.7	40.7	34	55
284	1304	24630	24648	50.8	52.6	3804	25403	25385	51.1	47.4	0.3	774	75.7	40.6	34	53.3
285	1305	9929	9946	50	50	3805	10605	10588	51.1	50	1	677	75.8	41.2	34	53.2
286	1306	24633	24651	50.1	52.6	3806	25403	25385	51.1	47.4	1	771	75.6	40.5	34	53.1
287	1307	11541	11560	50.1	45	3807	12253	12235	50.1	52.6	0	713	76.2	42.1	34	53.5
288	1308	24635	24653	50.5	52.6	3808	25403	25385	51.1	47.4	0.7	769	75.6	40.4	34	53.2
289	1309	9933	9952	50.9	45	3809	10608	10589	51	50	0.1	676	75.8	41.1	34	53.4
290	1310	24921	24938	50.4	50	3810	25548	25531	51.1	50	0.7	628	75.6	40.9	34	53.1
291	1311	7725	7743	50.8	47.4	3811	8188	8169	50.5	45	0.4	464	75.6	41.8	34	53.2
292	1312	28547	28568	53.5	45.5	3812	29301	29282	55.3	55	1.8	755	78.5	47.5	34	56.1
293	1313	28547	28568	53.5	45.5	3813	29306	29288	53.5	52.6	0	760	78.5	47.5	34	56.1
294	1314	28548	28568	50.5	42.9	3814	29298	29280	51.4	52.6	0.9	751	78.4	47.4	34	55.2
295	1315	28546	28567	55.1	50	3815	29301	29282	55.3	55	0.2	756	78.5	47.6	34	56.6
296	1316	28547	28567	52.9	47.6	3816	29298	29280	51.4	52.6	1.5	752	78.5	47.5	34	55.5
297	1317	28546	28565	52.2	50	3817	29298	29280	51.4	52.6	0.8	753	78.5	47.5	34	55.5
298	1318	28546	28565	52.2	50	3818	29306	29288	53.5	52.6	1.3	761	78.5	47.6	34	55.7
299	1319	28396	28416	52.4	47.6	3819	28672	28654	50.6	52.6	1.8	277	78.6	51.6	34	55.3
300	1320	28396	28415	51.2	45	3820	28671	28652	52.8	55	1.6	276	78.6	51.4	34	55.4
301	1321	28396	28415	51.2	45	3821	28671	28653	50.2	52.6	1	276	78.6	51.4	34	55.2
302	1322	12976	12995	51.1	45	3822	13545	13527	50.3	52.6	0.8	570	77.4	45.6	34	54.4
303	1323	16551	16568	51.1	50	3823	16711	16691	51	42.9	0.1	161	73.8	44.1	34	52.1
304	1324	28395	28414	51.5	45	3824	28672	28654	50.6	52.6	0.9	278	78.6	51.4	34	55.3
305	1325	16555	16572	50.3	50	3825	16780	16760	51.4	42.9	1.1	226	73.6	40.7	34	51.7
306	1326	28394	28413	51.8	45	3826	28671	28652	52.8	55	1	278	78.6	51.4	34	55.6
307	1327	28395	28413	50.2	42.1	3827	28671	28653	50.2	52.6	0	277	78.5	51.3	34	55.1
308	1328	7728	7746	51.7	52.6	3828	8049	8032	50.4	50	1.3	322	74.9	41.6	34	52.6
309	1329	28394	28412	51.1	47.4	3829	28671	28652	52.8	55	1.7	278	78.6	51.4	34	55.4
310	1330	28394	28412	51.1	47.4	3830	28671	28653	50.2	52.6	0.8	278	78.6	51.4	34	55.2
311	1331	11543	11562	50.4	40	3831	12257	12237	51.3	47.6	0.9	715	76.2	42	34	53.5
312	1332	28393	28411	52.9	52.6	3832	28671	28652	52.8	55	0.1	279	78.6	51.6	34	56
313	1333	28394	28411	50.3	50	3833	28671	28653	50.2	52.6	0	278	78.6	51.4	34	55.2
314	1334	4255	4276	51.7	45.5	3834	4710	4691	50.2	45	1.5	456	75.1	40.8	34	52.8
315	1335	12975	12994	52.1	45	3835	13545	13526	52.9	55	0.8	571	77.4	45.5	34	54.9
316	1336	9930	9948	51.5	52.6	3836	10608	10589	51	50	0.5	679	75.8	41.2	34	53.5
317	1337	27665	27686	51.4	40.9	3837	28411	28393	52.9	52.6	1.6	747	76.8	43.5	34	54.3
318	1338	27665	27686	51.4	40.9	3838	28415	28396	51.2	45	0.2	751	76.8	43.4	34	54.2
319	1339	11541	11561	50.9	42.9	3839	12257	12237	51.3	47.6	0.5	717	76.2	42	34	53.7
320	1340	27665	27685	50.7	42.9	3840	28415	28396	51.2	45	0.5	751	76.8	43.4	34	54.1
321	1341	11543	11562	50.4	40	3841	12253	12235	50.1	52.6	0.3	711	76.2	42.1	34	53.5

322	1342	11545	11563	50.8	47.4	3842	12254	12236	50.5	47.4	0.2	710	76.2	42.1	34	53.6
323	1343	27436	27455	52.7	45	3843	27542	27522	50.9	42.9	1.8	107	72	44.9	34	50.8
324	1344	27436	27455	52.7	45	3844	27546	27527	51.3	50	1.3	111	72.7	45.9	34	51.4
325	1345	27389	27407	50.6	47.4	3845	27541	27522	50.1	45	0.5	153	73.2	43.1	34	51.4
326	1346	27389	27407	50.6	47.4	3846	27546	27527	51.3	50	0.7	158	73.5	43.7	34	51.8
327	1347	27369	27392	57.2	50	3847	27468	27446	57.7	47.8	0.5	100	71.2	44	34	52.1
328	1348	27367	27389	55	47.8	3848	27466	27446	56.8	52.4	1.8	100	71.6	45	34	51.7
329	1349	11541	11560	50.1	45	3849	12257	12237	51.3	47.6	1.2	717	76.2	42	34	53.5
330	1350	7725	7742	50	50	3850	8188	8169	50.5	45	0.4	464	75.6	41.8	34	53
331	1351	2223	2243	50.2	42.9	3851	2672	2653	51.6	50	1.4	450	77	45.3	34	54.1
332	1352	9930	9949	52.2	50	3852	10608	10589	51	50	1.2	679	75.8	41.2	34	53.5
333	1353	9934	9953	50.7	50	3853	10455	10435	50.5	42.9	0.3	522	75.3	40.6	34	52.9
334	1354	2223	2243	50.2	42.9	3854	2672	2654	50.9	52.6	0.7	450	77	45.3	34	54.1
335	1355	3797	3815	50.9	47.4	3855	4445	4425	50.6	42.9	0.4	649	75.4	40.4	34	53.1
336	1356	9934	9953	50.7	50	3856	10455	10434	51.1	40.9	0.4	522	75.3	40.6	34	53
337	1357	18074	18093	50.3	45	3857	18697	18679	51.9	52.6	1.5	624	76.2	42.5	34	53.6
338	1358	12976	12994	50.3	47.4	3858	13545	13527	50.3	52.6	0.1	570	77.4	45.6	34	54.4
339	1359	12040	12057	50.6	50	3859	12498	12480	50	47.4	0.6	459	76.3	43.6	34	53.5
340	1360	12040	12057	50.6	50	3860	12257	12237	51.3	47.6	0.7	218	75.4	45.4	34	53.1
341	1361	11540	11557	50.4	50	3861	12257	12237	51.3	47.6	0.9	718	76.2	42.1	34	53.6
342	1362	12975	12993	51.4	47.4	3862	13545	13526	52.9	55	1.5	571	77.4	45.5	34	54.7
343	1363	12975	12993	51.4	47.4	3863	13545	13527	50.3	52.6	1.1	571	77.4	45.5	34	54.4
344	1364	11540	11560	53.2	47.6	3864	11983	11965	53	52.6	0.2	444	75.1	40.8	34	53.6
345	1365	12040	12057	50.6	50	3865	12253	12235	50.1	52.6	0.5	214	75.5	45.8	34	53
346	1366	12976	12995	51.1	45	3866	13545	13526	52.9	55	1.8	570	77.4	45.6	34	54.6
347	1367	13039	13057	51.1	52.6	3867	13314	13297	51	50	0.1	276	75.7	44.6	34	53.4
348	1368	27361	27380	52.4	55	3868	27463	27444	51.6	40	0.8	103	71.7	44.7	34	50.7
349	1369	27361	27380	52.4	55	3869	27463	27445	50.8	42.1	1.6	103	71.7	44.7	34	50.5
350	1370	27361	27380	52.4	55	3870	27464	27446	51.7	47.4	0.7	104	71.9	45.2	34	51
351	1371	25348	25365	50.4	50	3871	25645	25626	50.8	45	0.4	298	74.6	41.3	34	52.4
352	1372	9922	9941	51.3	50	3872	10449	10431	50.9	47.4	0.3	528	75.4	40.9	34	53.2
353	1373	13039	13057	51.1	52.6	3873	13323	13304	51.1	45	0	285	75.8	44.6	34	53.5
354	1374	12235	12253	50.1	52.6	3874	12412	12392	50	42.9	0.1	178	73.2	41.6	34	51.3
355	1375	3016	3036	50.2	42.9	3875	3185	3164	51	45.5	0.7	170	74.5	45.3	34	52.3
356	1376	13039	13057	51.1	52.6	3876	13326	13306	50.7	42.9	0.4	288	75.8	44.4	34	53.4
357	1377	7869	7889	52.5	47.6	3877	8050	8032	52	52.6	0.5	182	73.8	42.9	34	52.3
358	1378	26421	26441	51.5	42.9	3878	26655	26634	50.6	40.9	0.9	235	74.1	41.7	34	52.2
359	1379	26421	26441	51.5	42.9	3879	26657	26639	50.8	47.4	0.7	237	74.2	41.8	34	52.3
360	1380	26040	26061	56.4	54.5	3880	26183	26159	54.9	40	1.5	144	72	41	34	52
361	1381	26040	26061	56.4	54.5	3881	26183	26160	54.5	41.7	2	144	72	41	34	51.9
362	1382	26040	26061	56.4	54.5	3882	26184	26161	55.1	41.7	1.3	145	71.9	40.7	34	52
363	1383	12373	12391	50.8	47.4	3883	12724	12705	52.4	55	1.6	352	75.6	42.9	34	53.2
364	1384	26040	26061	56.4	54.5	3884	26589	26569	54.7	47.6	1.7	550	75.1	40	34	54.1
365	1385	26039	26058	54	55	3885	26183	26159	54.9	40	0.9	145	71.9	40.7	34	51.7
366	1386	26039	26058	54	55	3886	26183	26160	54.5	41.7	0.4	145	71.9	40.7	34	51.7
367	1387	26039	26058	54	55	3887	26183	26161	54	43.5	0	145	71.9	40.7	34	51.7
368	1388	26039	26058	54	55	3888	26184	26163	53	40.9	1	146	71.8	40.4	34	51.3
369	1389	26039	26057	52.6	52.6	3889	26174	26153	51	40.9	1.6	136	71.8	41.2	34	50.7
370	1390	10246	10266	50.4	47.6	3890	10605	10588	51.1	50	0.6	360	74.5	40.3	34	52.4
371	1391	3234	3254	51.1	47.6	3891	3497	3478	51.3	50	0.2	264	74.3	41.3	34	52.4
372	1392	26039	26057	52.6	52.6	3892	26183	26162	52.8	45.5	0.2	145	71.9	40.7	34	51.2

373	1393	11540	11557	50.4	50	3893	12253	12235	50.1	52.6	0.3	714	76.2	42.2	34	53.5
374	1394	3234	3254	51.1	47.6	3894	3500	3481	51.2	50	0.1	267	74.3	41.2	34	52.4
375	1395	3794	3812	52.9	52.6	3895	4445	4424	51.3	40.9	1.6	652	75.5	40.5	34	53.3
376	1396	3794	3812	52.9	52.6	3896	4446	4425	51.8	45.5	1.1	653	75.5	40.6	34	53.5
377	1397	3234	3254	51.1	47.6	3897	3646	3625	52	40.9	1	413	75.1	41.2	34	53
378	1398	3234	3254	51.1	47.6	3898	3647	3628	50.6	45	0.5	414	75.2	41.3	34	52.9
379	1399	3226	3245	51.7	55	3899	3497	3478	51.3	50	0.4	272	74.6	41.9	34	52.7
380	1400	3797	3815	50.9	47.4	3900	4444	4424	50.6	42.9	0.4	648	75.4	40.4	34	53.1
381	1401	3226	3245	51.7	55	3901	3500	3481	51.2	50	0.5	275	74.6	41.8	34	52.7
382	1402	16366	16384	50.3	52.6	3902	16780	16760	51.4	42.9	1.1	415	75.1	41	34	52.7
383	1403	25782	25806	53.5	40	3903	26183	26161	54	43.5	0.5	402	74.7	40.3	34	53.5
384	1404	16366	16385	52.9	55	3904	16780	16760	51.4	42.9	1.4	415	75.1	41	34	53.1
385	1405	16367	16386	51.4	50	3905	16781	16761	51.3	47.6	0.1	415	75.1	41	34	53
386	1406	12236	12256	51.2	42.9	3906	12992	12974	51.2	52.6	0	757	76.5	42.5	34	54
387	1407	16367	16386	51.4	50	3907	16777	16758	51.5	50	0.1	411	75	40.9	34	53
388	1408	16367	16386	51.4	50	3908	16711	16691	51	42.9	0.3	345	75.2	42	34	53
389	1409	3226	3245	51.7	55	3909	3503	3484	51.5	50	0.3	278	74.7	42.1	34	52.9
390	1410	16548	16566	54.9	52.6	3910	16782	16760	54.3	43.5	0.6	235	74	41.3	34	53.2
391	1411	16549	16567	54.9	52.6	3911	16782	16760	54.3	43.5	0.6	234	74	41.5	34	53.2
392	1412	25354	25372	50.9	52.6	3912	25645	25626	50.8	45	0.2	292	74.4	41.1	34	52.4
393	1413	16551	16568	51.1	50	3913	17038	17021	50.7	50	0.4	488	75.8	42.2	34	53.4
394	1414	25348	25366	51.2	47.4	3914	25645	25626	50.8	45	0.4	298	74.6	41.3	34	52.5
395	1415	16551	16568	51.1	50	3915	16780	16760	51.4	42.9	0.3	230	73.9	41.3	34	52.2
396	1416	7725	7743	50.8	47.4	3916	8049	8032	50.4	50	0.5	325	74.9	41.5	34	52.6
397	1417	29200	29224	54.2	40	3917	29299	29280	53.9	55	0.3	100	72.8	48	34	52.3
398	1418	29200	29224	54.2	40	3918	29301	29282	55.3	55	1.1	102	73	48	34	52.4
399	1419	29200	29223	53.7	41.7	3919	29299	29280	53.9	55	0.2	100	72.8	48	34	52.2
400	1420	29200	29223	53.7	41.7	3920	29301	29282	55.3	55	1.6	102	73	48	34	52.3
401	1421	29199	29222	54.6	41.7	3921	29301	29282	55.3	55	0.7	103	72.9	47.6	34	52.5
402	1422	29200	29222	53.2	43.5	3922	29299	29280	53.9	55	0.7	100	72.8	48	34	52
403	1423	29199	29221	54.1	43.5	3923	29301	29282	55.3	55	1.2	103	72.9	47.6	34	52.3
404	1424	29200	29221	52.6	45.5	3924	29299	29280	53.9	55	1.3	100	72.8	48	34	51.9
405	1425	18074	18093	50.3	45	3925	18239	18220	50	45	0.3	166	73.9	44	34	51.8
406	1426	18074	18093	50.3	45	3926	18238	18219	50.3	45	0	165	74	44.2	34	51.9
407	1427	1402	1426	54.1	40	3927	1774	1755	53.1	50	1	373	75.8	43.2	34	54.1
408	1428	18074	18094	51.1	42.9	3928	18697	18679	51.9	52.6	0.8	624	76.2	42.5	34	53.8
409	1429	18074	18094	51.1	42.9	3929	18239	18220	50	45	1	166	73.9	44	34	51.8
410	1430	18074	18094	51.1	42.9	3930	18238	18219	50.3	45	0.8	165	74	44.2	34	51.9
411	1431	3226	3245	51.7	55	3931	3504	3485	50.4	45	1.3	279	74.7	41.9	34	52.5
412	1432	18081	18099	51.2	52.6	3932	18662	18641	50.4	40.9	0.7	582	76.3	42.8	34	53.6
413	1433	7725	7742	50	50	3933	8049	8032	50.4	50	0.3	325	74.9	41.5	34	52.5
414	1434	29182	29205	54.6	41.7	3934	29301	29282	55.3	55	0.7	120	73.4	46.7	34	52.9
415	1435	4255	4276	51.7	45.5	3935	4711	4692	51.2	45	0.5	457	75.1	40.7	34	53
416	1436	29183	29204	50.4	40.9	3936	29298	29280	51.4	52.6	1.1	116	72.8	45.7	34	51.2
417	1437	3225	3243	50.9	52.6	3937	3497	3478	51.3	50	0.4	273	74.7	42.1	34	52.7
418	1438	29181	29202	53.9	45.5	3938	29301	29282	55.3	55	1.4	121	73.6	47.1	34	52.8
419	1439	29182	29202	51.2	42.9	3939	29298	29280	51.4	52.6	0.2	117	73.1	46.2	34	51.6
420	1440	29180	29199	50.1	40	3940	29298	29280	51.4	52.6	1.3	119	73.2	46.2	34	51.4
421	1441	28970	28993	53.3	41.7	3941	29301	29282	55.3	55	1.9	332	76.1	44.6	34	54.4
422	1442	28971	28993	51.9	43.5	3942	29298	29280	51.4	52.6	0.5	328	76.1	44.5	34	53.8
423	1443	4255	4276	51.7	45.5	3943	4711	4693	50.4	47.4	1.3	457	75.1	40.7	34	52.8
424	1444	12976	12996	51.8	42.9	3944	13545	13526	52.9	55	1.1	570	77.4	45.6	34	54.8

425	1445	28968	28989	51.5	45.5	3945	29306	29288	53.5	52.6	2	339	76.3	44.8	34	54
426	1446	3225	3243	50.9	52.6	3946	3500	3481	51.2	50	0.3	276	74.7	42	34	52.6
427	1447	3225	3243	50.9	52.6	3947	3503	3484	51.5	50	0.6	279	74.8	42.3	34	52.7
428	1448	3225	3243	50.9	52.6	3948	3504	3485	50.4	45	0.5	280	74.8	42.1	34	52.6
429	1449	28939	28961	55.2	47.8	3949	29301	29282	55.3	55	0.1	363	76.6	45.2	34	55.3
430	1450	28941	28961	51.6	42.9	3950	29306	29288	53.5	52.6	1.8	366	76.4	44.8	34	54.1
431	1451	8867	8886	50.7	50	3951	9252	9235	50.1	50	0.6	386	75.1	41.5	34	52.7
432	1452	28939	28960	54.7	50	3952	29301	29282	55.3	55	0.6	363	76.6	45.2	34	55.1
433	1453	28940	28960	52.4	47.6	3953	29306	29288	53.5	52.6	1	367	76.5	45	34	54.4
434	1454	28941	28960	50.9	45	3954	29298	29280	51.4	52.6	0.5	358	76.3	44.7	34	53.8
435	1455	3360	3380	51.4	42.9	3955	3494	3473	50.4	40.9	1	135	73.7	45.9	34	51.8
436	1456	19709	19730	51.3	40.9	3956	19916	19895	50.2	40.9	1	208	73.6	41.3	34	51.7
437	1457	12373	12391	50.8	47.4	3957	12994	12976	50.3	47.4	0.4	622	76.4	42.9	34	53.7
438	1458	19794	19813	50	50	3958	19921	19900	51.8	45.5	1.8	128	72.6	43.8	34	50.9
439	1459	3361	3381	50.5	42.9	3959	3494	3473	50.4	40.9	0.1	134	73.8	46.3	34	51.9
440	1460	12234	12252	50.6	47.4	3960	12992	12974	51.2	52.6	0.6	759	76.5	42.6	34	53.8
441	1461	12234	12252	50.6	47.4	3961	12994	12976	50.3	47.4	0.2	761	76.4	42.4	34	53.7
442	1462	3034	3053	50.3	50	3962	3647	3628	50.6	45	0.3	614	76.4	42.8	34	53.6
443	1463	8867	8887	52.3	47.6	3963	9254	9236	50.6	47.4	1.7	388	75.1	41.2	34	52.8
444	1464	12726	12746	51.3	47.6	3964	12994	12976	50.3	47.4	1	269	75.1	43.1	34	52.8
445	1465	12234	12252	50.6	47.4	3965	12998	12979	50.1	45	0.5	765	76.4	42.5	34	53.6
446	1466	9926	9944	50.5	52.6	3966	10605	10588	51.1	50	0.6	680	75.8	41.2	34	53.3
447	1467	3034	3053	50.3	50	3967	3646	3625	52	40.9	1.7	613	76.3	42.7	34	53.6
448	1468	12977	12996	50.2	40	3968	13545	13527	50.3	52.6	0	569	77.4	45.5	34	54.3
449	1469	19799	19817	52.2	52.6	3969	19909	19885	52.5	40	0.3	111	71.6	43.2	34	50.8
450	1470	8867	8887	52.3	47.6	3970	9246	9226	50.5	42.9	1.8	380	75	41.1	34	52.7
451	1471	8867	8887	52.3	47.6	3971	9342	9323	52.1	50	0.3	476	75.7	42	35	53.7
452	1472	10141	10160	51	45	3972	10608	10589	51	50	0	468	74.9	40.2	35	52.8
453	1473	3192	3213	51.8	45.5	3973	3494	3473	50.4	40.9	1.4	303	74.9	41.9	35	52.6
454	1474	3360	3379	50.7	45	3974	3647	3628	50.6	45	0.1	288	75.5	43.8	35	53.1
455	1475	27367	27385	51.4	52.6	3975	27566	27546	50.7	47.6	0.7	200	74.8	44.5	35	52.7
456	1476	10250	10274	51.6	40	3976	10605	10588	51.1	50	0.5	356	74.6	40.4	35	52.6
457	1477	27367	27385	51.4	52.6	3977	27568	27548	50.2	42.9	1.2	202	74.6	44.1	35	52.4
458	1478	27367	27385	51.4	52.6	3978	27571	27551	51.4	42.9	0	205	74.6	43.9	35	52.7
459	1479	8867	8887	52.3	47.6	3979	9376	9355	51	40.9	1.3	510	75.7	41.8	35	53.4
460	1480	27367	27385	51.4	52.6	3980	27576	27555	51	40.9	0.4	210	74.8	44.3	35	52.8
461	1481	27367	27385	51.4	52.6	3981	27579	27558	51.1	40.9	0.3	213	75	44.6	35	52.9
462	1482	18704	18724	50.8	47.6	3982	19215	19194	50.2	40.9	0.5	512	75.5	41.2	35	53
463	1483	18704	18724	50.8	47.6	3983	19217	19196	50.2	40.9	0.5	514	75.5	41.2	35	53
464	1484	18696	18715	51.7	50	3984	19215	19194	50.2	40.9	1.5	520	75.6	41.3	35	53.1
465	1485	27365	27384	52.6	50	3985	27464	27443	54	45.5	1.4	100	70.8	43	35	50.4
466	1486	18696	18715	51.7	50	3986	19217	19196	50.2	40.9	1.5	522	75.6	41.4	35	53.1
467	1487	3361	3381	50.5	42.9	3987	3646	3625	52	40.9	1.5	286	75.5	43.7	35	53.1
468	1488	3361	3381	50.5	42.9	3988	3647	3628	50.6	45	0.1	287	75.6	43.9	35	53.1
469	1489	3782	3801	51.3	50	3989	4445	4425	50.6	42.9	0.7	664	75.5	40.5	35	53.1
470	1490	13039	13058	51.8	50	3990	13155	13137	52.1	52.6	0.3	117	73.4	47	35	52
471	1491	3782	3801	51.3	50	3991	4444	4424	50.6	42.9	0.7	663	75.5	40.6	35	53.1
472	1492	13040	13059	50.9	50	3992	13747	13726	50.8	40.9	0.1	708	76.6	43.1	35	54
473	1493	2223	2243	50.2	42.9	3993	2747	2727	50	42.9	0.2	525	76.9	44.6	35	53.9
474	1494	9929	9946	50	50	3994	10449	10431	50.9	47.4	0.9	521	75.4	40.9	35	52.9
475	1495	18077	18097	51.5	47.6	3995	18702	18685	50.2	50	1.4	626	76.2	42.3	35	53.5

476	1496	3360	3379	50.7	45	3996	3646	3625	52	40.9	1.3	287	75.4	43.6	35	53.1
477	1497	26708	26731	54.2	41.7	3997	27463	27443	52.7	42.9	1.5	756	75.9	41.1	35	54
478	1498	26708	26731	54.2	41.7	3998	27464	27444	53	42.9	1.2	757	75.9	41.2	35	54.1
479	1499	26708	26731	54.2	41.7	3999	27464	27445	52.4	45	1.8	757	75.9	41.2	35	54
480	1500	4	22	52.3	52.6	4000	713	695	50.7	47.4	1.6	710	79	49	35	55.7
481	1501	26708	26727	50	45	4001	27462	27443	51.4	45	1.3	755	75.9	41.2	35	53.2
482	1502	3360	3380	51.4	42.9	4002	3646	3625	52	40.9	0.6	287	75.4	43.6	35	53.3
483	1503	26708	26727	50	45	4003	27463	27445	50.8	42.1	0.8	756	75.9	41.1	35	53.2
484	1504	988	1006	52.2	52.6	4004	1493	1474	50.8	45	1.5	506	76.5	43.7	35	53.9
485	1505	12352	12375	52.9	41.7	4005	12993	12975	51.4	47.4	1.5	642	76.5	43	35	54
486	1506	3360	3380	51.4	42.9	4006	3647	3628	50.6	45	0.8	288	75.5	43.8	35	53.1
487	1507	18074	18094	51.1	42.9	4007	18702	18685	50.2	50	0.9	629	76.2	42.3	35	53.5
488	1508	3360	3380	51.4	42.9	4008	3650	3631	53.1	50	1.7	291	75.6	44	35	53.5
489	1509	8374	8395	52.4	45.5	4009	8928	8911	51.9	50	0.6	555	75.1	40	35	53.2
490	1510	9929	9946	50	50	4010	10449	10428	51.9	40.9	1.9	521	75.4	40.9	35	52.9
491	1511	26421	26441	51.5	42.9	4011	27132	27111	50.3	40.9	1.2	712	77.1	44.2	35	54.2
492	1512	10250	10274	51.6	40	4012	10356	10336	52.4	47.6	0.8	107	70.8	42.1	35	50.2
493	1513	18074	18093	50.3	45	4013	18702	18685	50.2	50	0.2	629	76.2	42.3	35	53.5
494	1514	18017	18036	54.8	55	4014	18220	18202	54.8	52.6	0	204	74.3	43.1	35	53.5
495	1515	18017	18036	54.8	55	4015	18225	18206	53.7	50	1.1	209	74.3	43.1	35	53.2
496	1516	18017	18036	54.8	55	4016	18232	18210	54.4	47.8	0.4	216	74.6	43.5	35	53.6
497	1517	18017	18036	54.8	55	4017	18234	18214	53.4	47.6	1.4	218	74.7	43.6	35	53.4
498	1518	18017	18036	54.8	55	4018	18235	18215	54.2	52.4	0.6	219	74.8	43.8	35	53.7
499	1519	18017	18036	54.8	55	4019	18443	18424	55.9	55	1.1	427	76	43.1	35	54.7
500	1520	18012	18031	53.2	55	4020	18220	18202	54.8	52.6	1.7	209	74.5	43.5	35	53.2
501	1521	18013	18031	50.6	52.6	4021	18223	18206	51.8	50	1.1	211	74.4	43.1	35	52.4
502	1522	18013	18031	50.6	52.6	4022	18231	18210	52.2	45.5	1.6	219	74.6	43.4	35	52.5
503	1523	18013	18031	50.6	52.6	4023	18233	18214	52	50	1.4	221	74.8	43.9	35	52.7
504	1524	18013	18031	50.6	52.6	4024	18233	18215	51.3	52.6	0.7	221	74.8	43.9	35	52.7
505	1525	18013	18031	50.6	52.6	4025	18662	18641	50.4	40.9	0.2	650	76.3	42.6	35	53.7
506	1526	18009	18029	53.3	52.4	4026	18220	18202	54.8	52.6	1.6	212	74.5	43.4	35	53.2
507	1527	18011	18029	51.3	52.6	4027	18223	18206	51.8	50	0.5	213	74.4	43.2	35	52.6
508	1528	18011	18029	51.3	52.6	4028	18231	18210	52.2	45.5	0.9	221	74.7	43.4	35	52.8
509	1529	18011	18029	51.3	52.6	4029	18233	18214	52	50	0.7	223	74.9	43.9	35	52.9
510	1530	18011	18029	51.3	52.6	4030	18233	18215	51.3	52.6	0	223	74.9	43.9	35	52.9
511	1531	16374	16397	52.8	41.7	4031	16774	16751	53.6	41.7	0.8	401	75	40.9	35	53.4
512	1532	16378	16397	50.4	45	4032	16780	16760	51.4	42.9	1	403	75	40.9	35	52.7
513	1533	2223	2243	50.2	42.9	4033	2997	2976	51.4	40.9	1.2	775	76.7	43.1	35	53.9
514	1534	2428	2447	51.5	50	4034	3082	3058	52.3	40	0.8	655	76.3	42.6	35	54
515	1535	16548	16566	54.9	52.6	4035	16774	16751	53.6	41.7	1.3	227	73.9	41.4	35	52.9
516	1536	16367	16386	51.4	50	4036	16774	16752	52.2	43.5	0.8	408	75	40.9	35	53
517	1537	3230	3249	50.1	45	4037	3497	3478	51.3	50	1.2	268	74.4	41.4	35	52.2
518	1538	8221	8240	52.4	50	4038	8920	8901	53.4	50	1	700	75.3	40	35	53.6
519	1539	3232	3252	51.1	47.6	4039	3500	3481	51.2	50	0.1	269	74.5	41.6	35	52.5
520	1540	3232	3252	51.1	47.6	4040	3497	3478	51.3	50	0.2	266	74.5	41.7	35	52.6
521	1541	16367	16386	51.4	50	4041	17111	17090	51.1	40.9	0.3	745	76.3	42.1	35	53.8
522	1542	16366	16385	52.9	55	4042	16774	16751	53.6	41.7	0.8	409	75.1	41.1	35	53.5
523	1543	9930	9948	51.5	52.6	4043	10670	10649	51.3	40.9	0.2	741	75.8	40.9	35	53.5
524	1544	12370	12388	50.1	47.4	4044	12996	12977	50.2	40	0.2	627	76.4	42.7	35	53.6
525	1545	25354	25372	50.9	52.6	4045	25650	25631	51.3	45	0.4	297	74.5	41.1	35	52.5
526	1546	25354	25372	50.9	52.6	4046	25651	25634	50.4	50	0.5	298	74.6	41.3	35	52.4

527	1547	25354	25372	50.9	52.6	4047	25772	25753	51.9	50	1	419	74.8	40.3	35	52.8
528	1548	1402	1422	50.2	42.9	4048	1501	1482	50.5	45	0.3	100	72	46	35	50.6
529	1549	25354	25372	50.9	52.6	4049	25831	25809	51.4	43.5	0.5	478	75	40.2	35	52.8
530	1550	3797	3815	50.9	47.4	4050	4434	4416	51.5	52.6	0.5	638	75.4	40.3	35	53.1
531	1551	25354	25372	50.9	52.6	4051	25831	25810	50.7	45.5	0.2	478	75	40.2	35	52.8
532	1552	3797	3815	50.9	47.4	4052	4435	4417	50.5	52.6	0.5	639	75.4	40.4	35	53
533	1553	24481	24500	50.1	45	4053	24938	24921	50.4	50	0.3	458	75.6	41.9	35	53.1
534	1554	25348	25366	51.2	47.4	4054	25831	25809	51.4	43.5	0.2	484	75	40.3	35	53
535	1555	25348	25366	51.2	47.4	4055	25831	25810	50.7	45.5	0.4	484	75	40.3	35	52.8
536	1556	24419	24440	52.3	45.5	4056	25080	25062	53.5	52.6	1.2	662	75.7	41.1	35	53.8
537	1557	24420	24440	50.8	42.9	4057	24527	24508	50.5	45	0.3	108	70.7	41.7	35	49.8
538	1558	25348	25365	50.4	50	4058	25650	25631	51.3	45	0.9	303	74.6	41.3	35	52.4
539	1559	25348	25365	50.4	50	4059	25651	25634	50.4	50	0.1	304	74.7	41.4	35	52.5
540	1560	25348	25365	50.4	50	4060	25831	25809	51.4	43.5	1	484	75	40.3	35	52.7
541	1561	25348	25365	50.4	50	4061	25831	25810	50.7	45.5	0.3	484	75	40.3	35	52.7
542	1562	28618	28636	52.5	52.6	4062	29298	29280	51.4	52.6	1.1	681	78.3	47.3	35	55.3
543	1563	8867	8887	52.3	47.6	4063	9317	9297	50.5	42.9	1.8	451	75.5	41.7	35	53.1
544	1564	28820	28838	53.7	52.6	4064	29301	29282	55.3	55	1.6	482	77.1	45.4	35	55.2
545	1565	27365	27385	53.2	47.6	4065	27464	27443	54	45.5	0.8	100	70.8	43	35	50.6
546	1566	28820	28839	54.3	50	4066	29306	29288	53.5	52.6	0.8	487	77.1	45.4	35	55.1
547	1567	28820	28839	54.3	50	4067	29301	29282	55.3	55	1	482	77.1	45.4	35	55.4
548	1568	28821	28840	51.8	45	4068	29306	29288	53.5	52.6	1.7	486	77.1	45.3	35	54.6
549	1569	27370	27389	50.1	45	4069	27675	27656	50	40	0.1	306	74.2	40.2	35	52
550	1570	28820	28840	54.8	47.6	4070	29301	29282	55.3	55	0.4	482	77.1	45.4	35	55.5
551	1571	27370	27389	50.1	45	4071	27674	27654	51.9	42.9	1.8	305	74.2	40.3	35	52.1
552	1572	2429	2447	50.2	47.4	4072	3188	3167	50.2	40.9	0	760	76.6	42.9	35	53.8
553	1573	27375	27392	50	50	4073	27675	27656	50	40	0	301	74.1	40.2	35	52
554	1574	27375	27392	50	50	4074	27674	27654	51.9	42.9	1.9	300	74.2	40.3	35	52
555	1575	19795	19814	50.4	45	4075	19916	19895	50.2	40.9	0.2	122	71.8	42.6	35	50.5
556	1576	3168	3189	51	45.5	4076	3646	3625	52	40.9	1.1	479	75.7	42	35	53.4
557	1577	3168	3189	51	45.5	4077	3647	3628	50.6	45	0.4	480	75.8	42.1	35	53.3
558	1578	18011	18029	51.3	52.6	4078	18662	18641	50.4	40.9	0.9	652	76.3	42.6	35	53.7
559	1579	985	1004	51.1	50	4079	1493	1474	50.8	45	0.3	509	76.5	43.6	35	53.9
560	1580	12965	12985	51.2	42.9	4080	13547	13528	50.2	45	0.9	583	77.3	45.3	35	54.3
561	1581	2427	2445	52.1	52.6	4081	3188	3167	50.2	40.9	1.9	762	76.7	43	35	53.8
562	1582	3360	3381	52.1	40.9	4082	3650	3631	53.1	50	1	291	75.6	44	35	53.7
563	1583	12726	12746	51.3	47.6	4083	12911	12892	50.5	50	0.8	186	73.5	41.9	35	51.7
564	1584	19800	19817	50.4	50	4084	19917	19896	50.9	45.5	0.5	118	71.9	43.2	35	50.6
565	1585	1402	1426	54.1	40	4085	1501	1478	54.6	41.7	0.5	100	72	46	35	51.8
566	1586	2427	2445	52.1	52.6	4086	3082	3058	52.3	40	0.2	656	76.4	42.7	35	54.2
567	1587	8867	8887	52.3	47.6	4087	9257	9238	50.5	45	1.8	391	75.1	41.2	35	52.8
568	1588	8867	8887	52.3	47.6	4088	9249	9231	50.8	47.4	1.5	383	75.2	41.5	35	53
569	1589	8374	8394	51	42.9	4089	8928	8911	51.9	50	0.8	555	75.1	40	35	53
570	1590	8867	8887	52.3	47.6	4090	9249	9230	51.5	45	0.8	383	75.2	41.5	35	53.2
571	1591	28964	28984	54.3	52.4	4091	29301	29282	55.3	55	1	338	76.5	45.3	35	54.9
572	1592	8867	8887	52.3	47.6	4092	9249	9229	53	47.6	0.6	383	75.2	41.5	35	53.4
573	1593	12962	12980	50.7	47.4	4093	13547	13528	50.2	45	0.5	586	77.4	45.4	35	54.3
574	1594	9931	9950	50.2	45	4094	10605	10588	51.1	50	0.9	675	75.8	41.2	35	53.2
575	1595	19801	19819	53.2	52.6	4095	19918	19896	52.2	43.5	1	118	71.9	43.2	35	51.1
576	1596	9055	9079	52.8	40	4096	9376	9355	51	40.9	1.8	322	75.1	42.2	35	53
577	1597	19878	19899	50.5	40.9	4097	20033	20016	50.4	50	0.1	156	73.4	43.6	35	51.6

578	1598	17608	17628	50.9	42.9	4098	18233	18214	52	50	1.1	626	75.3	40.3	35	53.1
579	1599	17608	17627	50.2	45	4099	18233	18214	52	50	1.8	626	75.3	40.3	35	52.9
580	1600	29179	29199	51.4	42.9	4100	29358	29339	52.8	50	1.4	180	74.8	45.6	35	52.9
581	1601	29182	29202	51.2	42.9	4101	29358	29339	52.8	50	1.6	177	74.6	45.2	35	52.7
582	1602	4	22	52.3	52.6	4102	253	233	51.8	47.6	0.5	250	76.2	46.4	35	54
583	1603	8221	8240	52.4	50	4103	8920	8902	52.8	52.6	0.3	700	75.3	40	35	53.6
584	1604	16554	16572	53.7	52.6	4104	16774	16751	53.6	41.7	0.1	221	73.7	41.2	35	52.8
585	1605	16555	16572	50.3	50	4105	16711	16691	51	42.9	0.7	157	73.4	43.3	35	51.6
586	1606	29186	29205	50.1	40	4106	29412	29393	50.3	45	0.3	227	75.4	44.9	35	52.9
587	1607	2429	2447	50.2	47.4	4107	3052	3033	50.3	50	0.1	624	76.3	42.6	35	53.6
588	1608	29182	29205	54.6	41.7	4108	29358	29339	52.8	50	1.7	177	74.6	45.2	35	53.2
589	1609	4	22	52.3	52.6	4109	255	235	51.3	47.6	1	252	76.3	46.4	35	53.9
590	1610	3230	3249	50.1	45	4110	3500	3481	51.2	50	1.1	271	74.4	41.3	35	52.2
591	1611	13040	13059	50.9	50	4111	13177	13156	50.4	40.9	0.5	138	73.7	45.7	35	51.8
592	1612	16551	16568	51.1	50	4112	17039	17022	51.4	50	0.3	489	75.8	42.1	35	53.5
593	1613	19995	20012	50.4	50	4113	20615	20597	50.6	47.4	0.2	621	75.3	40.1	35	52.9
594	1614	19995	20013	51.8	52.6	4114	20615	20597	50.6	47.4	1.2	621	75.3	40.1	35	53
595	1615	12370	12388	50.1	47.4	4115	12993	12975	51.4	47.4	1.3	624	76.4	42.9	35	53.6
596	1616	8374	8393	51.2	45	4116	8928	8911	51.9	50	0.7	555	75.1	40	35	53
597	1617	24174	24194	50.9	42.9	4117	24936	24919	51.8	50	0.8	763	75.8	41	35	53.5
598	1618	24179	24198	51	45	4118	24936	24919	51.8	50	0.7	758	75.8	41	35	53.5
599	1619	7679	7698	50.6	50	4119	8049	8032	50.4	50	0.2	371	75.4	42.3	35	53
600	1620	13177	13197	50.3	42.9	4120	13320	13300	51.4	47.6	1.1	144	73.2	43.8	35	51.4
601	1621	24179	24200	53.3	40.9	4121	24934	24913	53.4	45.5	0.2	756	75.8	41	35	54.2
602	1622	9927	9945	50.8	52.6	4122	10670	10649	51.3	40.9	0.5	744	75.7	40.9	35	53.4
603	1623	2427	2445	52.1	52.6	4123	3052	3033	50.3	50	1.8	626	76.4	42.8	35	53.6
604	1624	24418	24436	50	47.4	4124	24527	24507	51	42.9	1	110	71.3	42.7	35	50
605	1625	24417	24436	52.6	50	4125	24517	24494	53.2	41.7	0.6	101	71.1	43.6	35	50.6
606	1626	8375	8396	51.8	45.5	4126	8929	8911	53.4	52.6	1.6	555	75.1	40	35	53.2
607	1627	24418	24439	52.9	45.5	4127	25080	25062	53.5	52.6	0.6	663	75.8	41.2	35	54
608	1628	18074	18094	51.1	42.9	4128	18662	18641	50.4	40.9	0.6	589	76.2	42.6	36	53.6
609	1629	18074	18094	51.1	42.9	4129	18632	18611	50.2	40.9	0.9	559	76.2	42.8	36	53.5
610	1630	13231	13251	50.1	42.9	4130	13545	13527	50.3	52.6	0.2	315	77	47	36	54
611	1631	7400	7417	50.2	50	4131	8188	8169	50.5	45	0.3	789	76.4	42.2	36	53.6
612	1632	3792	3811	54	55	4132	4446	4424	52.4	43.5	1.6	655	75.5	40.6	36	53.7
613	1633	25782	25805	52.1	41.7	4133	26182	26161	51.2	40.9	0.9	401	74.7	40.1	36	52.7
614	1634	13230	13251	52.4	45.5	4134	13545	13526	52.9	55	0.5	316	77.1	47.2	36	54.8
615	1635	985	1004	51.1	50	4135	1480	1462	51.6	47.4	0.5	496	76.4	43.5	36	53.9
616	1636	7400	7417	50.2	50	4136	8049	8032	50.4	50	0.2	650	76.1	42.2	36	53.5
617	1637	13176	13197	52.7	45.5	4137	13545	13526	52.9	55	0.2	370	77	46.2	36	54.8
618	1638	25782	25806	53.5	40	4138	26183	26162	52.8	45.5	0.7	402	74.7	40.3	36	53.3
619	1639	13176	13196	51.4	47.6	4139	13547	13528	50.2	45	1.2	372	76.9	46	36	54
620	1640	12938	12956	50.1	47.4	4140	13155	13138	50.4	50	0.3	218	75.4	45.4	36	52.9
621	1641	18080	18099	53	50	4141	18712	18693	54.8	55	1.9	633	76.3	42.7	36	54.4
622	1642	9140	9159	50.1	45	4142	9375	9354	50.4	40.9	0.3	236	74.6	42.8	36	52.3
623	1643	7725	7742	50	50	4143	8054	8035	50.4	50	0.4	330	75	41.8	36	52.6
624	1644	9922	9941	51.3	50	4144	10455	10435	50.5	42.9	0.8	534	75.3	40.6	36	52.9
625	1645	12938	12957	50.9	45	4145	13155	13138	50.4	50	0.5	218	75.4	45.4	36	53
626	1646	12366	12384	51.7	52.6	4146	12996	12977	50.2	40	1.4	631	76.4	42.8	36	53.6
627	1647	7617	7636	50.9	50	4147	8049	8032	50.4	50	0.6	433	75.7	42.3	36	53.2
628	1648	2671	2692	52.1	40.9	4148	3188	3167	50.2	40.9	2	518	75.6	41.5	36	53.1

629	1649	26039	26057	52.6	52.6	4149	26183	26164	51	45	1.6	145	71.9	40.7	36	50.8
630	1650	11540	11557	50.4	50	4150	11727	11708	50.4	45	0.1	188	73.1	41	36	51.4
631	1651	12962	12980	50.7	47.4	4151	13545	13527	50.3	52.6	0.4	584	77.4	45.5	36	54.4
632	1652	12961	12980	53.2	50	4152	13545	13526	52.9	55	0.4	585	77.5	45.6	36	55.2
633	1653	9055	9079	52.8	40	4153	9369	9350	51.5	50	1.4	315	75.3	42.9	36	53.3
634	1654	12965	12985	51.2	42.9	4154	13545	13527	50.3	52.6	0.9	581	77.4	45.4	36	54.3
635	1655	26039	26058	54	55	4155	26693	26674	54.8	55	0.8	655	75.7	41.1	36	54.3
636	1656	26039	26058	54	55	4156	26692	26673	52.6	50	1.4	654	75.7	41	36	53.8
637	1657	26039	26058	54	55	4157	26688	26669	52.1	45	2	650	75.6	40.8	36	53.6
638	1658	26039	26058	54	55	4158	26684	26666	53.4	52.6	0.6	646	75.6	40.9	36	54.1
639	1659	26039	26058	54	55	4159	26683	26665	52.7	52.6	1.4	645	75.6	40.9	36	53.8
640	1660	12965	12985	51.2	42.9	4160	13545	13526	52.9	55	1.7	581	77.4	45.4	36	54.6
641	1661	26039	26058	54	55	4161	26183	26162	52.8	45.5	1.2	145	71.9	40.7	36	51.3
642	1662	9055	9079	52.8	40	4162	9365	9347	53	52.6	0.2	311	75.3	42.8	36	53.6
643	1663	19795	19814	50.4	45	4163	19922	19902	50	42.9	0.4	128	72.2	43	36	50.7
644	1664	12965	12988	54	41.7	4164	13545	13526	52.9	55	1.2	581	77.4	45.4	36	55.1
645	1665	26040	26061	56.4	54.5	4165	26693	26674	54.8	55	1.6	654	75.7	41.1	36	54.6
646	1666	26040	26061	56.4	54.5	4166	26693	26673	55.3	52.4	1.1	654	75.7	41.1	36	54.7
647	1667	26040	26061	56.4	54.5	4167	26690	26669	56.3	50	0.1	651	75.7	41	36	55
648	1668	26040	26061	56.4	54.5	4168	26685	26666	54.8	55	1.6	646	75.7	41	36	54.5
649	1669	26040	26061	56.4	54.5	4169	26685	26665	55.3	52.4	1.1	646	75.7	41	36	54.7
650	1670	18011	18031	54.5	52.4	4170	18443	18424	55.9	55	1.4	433	76.1	43.2	36	54.7
651	1671	7876	7895	51.5	45	4171	8049	8032	50.4	50	1.2	174	73.2	42	36	51.5
652	1672	3230	3249	50.1	45	4172	3646	3625	52	40.9	1.9	417	75.2	41.2	36	52.8
653	1673	19795	19814	50.4	45	4173	19920	19899	50.2	40.9	0.3	126	72.1	42.9	36	50.6
654	1674	12366	12384	51.7	52.6	4174	12993	12975	51.4	47.4	0.3	628	76.5	43	36	54
655	1675	19793	19814	54	50	4175	20544	20524	52.3	47.6	1.7	752	75.4	40	36	53.6
656	1676	12366	12384	51.7	52.6	4176	12911	12892	50.5	50	1.2	546	76.1	42.5	36	53.5
657	1677	7728	7746	51.7	52.6	4177	8188	8168	50.4	42.9	1.3	461	75.6	41.9	36	53.1
658	1678	26421	26441	51.5	42.9	4178	27084	27063	51.6	40.9	0.2	664	77.3	45	36	54.7
659	1679	9929	9946	50	50	4179	10455	10434	51.1	40.9	1.1	527	75.3	40.6	36	52.8
660	1680	26421	26441	51.5	42.9	4180	27083	27062	50.7	40.9	0.8	663	77.4	45.1	36	54.5
661	1681	12236	12256	51.2	42.9	4181	12999	12980	50.6	40	0.6	764	76.4	42.4	36	53.8
662	1682	26421	26441	51.5	42.9	4182	26694	26677	51.4	50	0	274	74.9	42.7	36	53
663	1683	9929	9946	50	50	4183	10183	10166	50.9	50	0.8	255	75.3	43.9	36	52.8
664	1684	12234	12252	50.6	47.4	4184	13000	12981	51.1	45	0.5	767	76.4	42.5	36	53.8
665	1685	8868	8889	50.4	40.9	4185	9254	9236	50.6	47.4	0.2	387	75	41.1	36	52.7
666	1686	9130	9150	51.3	42.9	4186	9597	9577	50.3	42.9	1	468	75.4	41.2	36	52.9
667	1687	9935	9955	50.4	42.9	4187	10605	10588	51.1	50	0.7	671	75.8	41.1	36	53.2
668	1688	26421	26441	51.5	42.9	4188	26587	26569	52	47.4	0.5	167	72.3	40.1	36	51.2
669	1689	9130	9150	51.3	42.9	4189	9597	9576	51	40.9	0.3	468	75.4	41.2	36	53.2
670	1690	26708	26727	50	45	4190	27466	27449	51	50	0.9	759	76	41.4	36	53.3
671	1691	9130	9150	51.3	42.9	4191	9375	9354	50.4	40.9	0.9	246	74.7	42.7	36	52.5
672	1692	10246	10266	50.4	47.6	4192	10608	10589	51	50	0.5	363	74.5	40.2	36	52.4
673	1693	9924	9944	53.1	52.4	4193	10449	10425	54.6	40	1.5	526	75.4	40.9	36	53.8
674	1694	12366	12384	51.7	52.6	4194	12911	12891	51.2	47.6	0.5	546	76.1	42.5	36	53.7
675	1695	26708	26731	54.2	41.7	4195	27466	27448	52.3	52.6	1.9	759	76	41.4	36	54
676	1696	8867	8888	52.7	45.5	4196	9107	9086	51.6	45.5	1.1	241	74.1	41.5	36	52.5
677	1697	9131	9151	50.4	42.9	4197	9597	9577	50.3	42.9	0.1	467	75.4	41.3	36	53
678	1698	9131	9151	50.4	42.9	4198	9597	9576	51	40.9	0.6	467	75.4	41.3	36	53
679	1699	10242	10265	51.2	41.7	4199	10608	10589	51	50	0.3	367	74.5	40.1	36	52.5

680	1700	27361	27380	52.4	55	4200	27468	27451	51.1	50	1.3	108	72.3	45.4	36	51
681	1701	27361	27380	52.4	55	4201	27467	27450	52.1	50	0.3	107	72.4	45.8	36	51.4
682	1702	27361	27380	52.4	55	4202	27466	27449	51	50	1.4	106	72.5	46.2	36	51.1
683	1703	9926	9944	50.5	52.6	4203	10449	10428	51.9	40.9	1.4	524	75.4	40.8	36	53
684	1704	9926	9944	50.5	52.6	4204	10449	10431	50.9	47.4	0.5	524	75.4	40.8	36	53
685	1705	19802	19820	53	52.6	4205	19922	19901	51.5	45.5	1.4	121	72.3	43.8	36	51.2
686	1706	27361	27380	52.4	55	4206	27462	27443	51.4	45	1	102	71.8	45.1	36	50.8
687	1707	10140	10159	52.4	50	4207	10605	10588	51.1	50	1.3	466	75	40.3	36	52.9
688	1708	16366	16384	50.3	52.6	4208	16777	16758	51.5	50	1.2	412	75.1	41	36	52.8
689	1709	16366	16385	52.9	55	4209	16781	16761	51.3	47.6	1.6	416	75.1	41.1	36	53.1
690	1710	985	1008	56.1	50	4210	1484	1464	54.3	47.6	1.8	500	76.4	43.6	36	54.9
691	1711	16366	16385	52.9	55	4211	16777	16758	51.5	50	1.4	412	75.1	41	36	53.1
692	1712	27366	27384	52.2	52.6	4212	27466	27448	52.3	52.6	0.1	101	71.5	44.6	36	50.8
693	1713	985	1008	56.1	50	4213	1483	1462	54.3	45.5	1.8	499	76.4	43.5	36	54.8
694	1714	2823	2844	50.4	45.5	4214	3052	3033	50.3	50	0.2	230	74.1	41.7	36	52
695	1715	3224	3242	50.5	52.6	4215	3504	3485	50.4	45	0.1	281	74.7	42	36	52.5
696	1716	8867	8886	50.7	50	4216	9310	9291	51.2	45	0.5	444	75.4	41.4	36	53.1
697	1717	8867	8886	50.7	50	4217	9254	9236	50.6	47.4	0.1	388	75.1	41.2	36	52.8
698	1718	9349	9367	51.7	52.6	4218	9989	9968	51	40.9	0.7	641	75.4	40.4	36	53.2
699	1719	8867	8887	52.3	47.6	4219	9369	9350	51.5	50	0.8	503	75.8	42.1	36	53.6
700	1720	8867	8887	52.3	47.6	4220	9341	9322	51.1	50	1.2	475	75.7	41.9	36	53.4
701	1721	9926	9944	50.5	52.6	4221	10608	10589	51	50	0.5	683	75.8	41.1	36	53.3
702	1722	7725	7742	50	50	4222	8190	8172	50.3	47.4	0.3	466	75.6	41.8	36	53
703	1723	9131	9151	50.4	42.9	4223	9375	9354	50.4	40.9	0	245	74.7	42.9	36	52.5
704	1724	3055	3075	51.8	47.6	4224	3494	3473	50.4	40.9	1.4	440	76	43	36	53.4
705	1725	7725	7742	50	50	4225	8189	8170	50.6	50	0.6	465	75.6	41.9	36	53.1
706	1726	2823	2844	50.4	45.5	4226	3056	3038	50.8	52.6	0.3	234	74.2	41.9	36	52.2
707	1727	12370	12388	50.1	47.4	4227	13155	13138	50.4	50	0.3	786	76.8	43.4	36	53.9
708	1728	3055	3075	51.8	47.6	4228	3209	3189	50.5	47.6	1.3	155	74.1	45.2	36	52.1
709	1729	8867	8887	52.3	47.6	4229	9340	9319	50.8	45.5	1.6	474	75.6	41.8	36	53.3
710	1730	27367	27385	51.4	52.6	4230	27466	27448	52.3	52.6	0.9	100	71.6	45	36	50.6
711	1731	14951	14975	52.2	40	4231	15146	15129	50.3	50	1.9	196	73.2	40.8	36	51.4
712	1732	8867	8887	52.3	47.6	4232	9311	9292	50.7	50	1.6	445	75.4	41.6	36	53.1
713	1733	12234	12252	50.6	47.4	4233	12999	12980	50.6	40	0	766	76.4	42.4	36	53.8
714	1734	3055	3076	52.4	45.5	4234	3495	3473	51.8	43.5	0.6	441	76	43.1	36	53.9
715	1735	8867	8887	52.3	47.6	4235	9109	9087	50.5	43.5	1.8	243	74	41.2	36	52.1
716	1736	3055	3076	52.4	45.5	4236	3209	3189	50.5	47.6	2	155	74.1	45.2	36	52.1
717	1737	2671	2692	52.1	40.9	4237	3053	3034	50.3	50	1.8	383	74.7	40.5	36	52.5
718	1738	16981	17000	51.3	50	4238	17501	17481	51.2	42.9	0.1	521	75.9	42.2	36	53.6
719	1739	3796	3814	50.8	52.6	4239	4444	4424	50.6	42.9	0.2	649	75.5	40.5	36	53.1
720	1740	3796	3814	50.8	52.6	4240	4445	4425	50.6	42.9	0.2	650	75.5	40.5	36	53.1
721	1741	27382	27401	50.8	45	4241	27546	27527	51.3	50	0.6	165	73.7	43.6	36	51.9
722	1742	27382	27401	50.8	45	4242	27541	27522	50.1	45	0.6	160	73.4	43.1	36	51.5
723	1743	27383	27403	50.3	42.9	4243	27546	27527	51.3	50	1.1	164	73.5	43.3	36	51.7
724	1744	27383	27403	50.3	42.9	4244	27541	27522	50.1	45	0.1	159	73.2	42.8	36	51.4
725	1745	17789	17811	52.9	43.5	4245	18220	18202	54.8	52.6	1.9	432	74.9	40.5	36	53.4
726	1746	17791	17813	52.9	43.5	4246	18220	18202	54.8	52.6	1.9	430	74.9	40.5	36	53.4
727	1747	18004	18023	51.1	50	4247	18233	18215	51.3	52.6	0.2	230	75	43.9	36	52.9
728	1748	18004	18023	51.1	50	4248	18231	18210	52.2	45.5	1.1	228	74.7	43.4	36	52.8
729	1749	27437	27456	50.2	40	4249	27546	27527	51.3	50	1.1	110	72.4	45.5	36	50.8
730	1750	27437	27456	50.2	40	4250	27541	27522	50.1	45	0.1	105	71.8	44.8	36	50.4

731	1751	18004	18023	51.1	50	4251	18223	18206	51.8	50	0.6	220	74.5	43.2	36	52.6
732	1752	12233	12251	51.1	52.6	4252	12994	12976	50.3	47.4	0.8	762	76.5	42.5	36	53.7
733	1753	7869	7889	52.5	47.6	4253	8192	8172	50.9	42.9	1.6	324	75.2	42.3	36	53
734	1754	3224	3242	50.5	52.6	4254	3503	3484	51.5	50	0.9	280	74.8	42.1	36	52.6
735	1755	3224	3242	50.5	52.6	4255	3500	3481	51.2	50	0.6	277	74.6	41.9	36	52.5
736	1756	3224	3242	50.5	52.6	4256	3497	3478	51.3	50	0.8	274	74.6	42	36	52.5
737	1757	1	22	54.8	50	4257	204	185	56.6	55	1.8	204	75.1	45.1	36	54.1
738	1758	9140	9159	50.1	45	4258	9597	9576	51	40.9	0.9	458	75.3	41.3	36	52.9
739	1759	28179	28200	50.8	40.9	4259	28671	28653	50.2	52.6	0.6	493	79.7	51.7	36	56
740	1760	9140	9159	50.1	45	4260	9560	9540	51.6	42.9	1.5	421	75.2	41.3	36	52.8
741	1761	7728	7746	51.7	52.6	4261	8189	8170	50.6	50	1.1	462	75.7	42	36	53.3
742	1762	9140	9159	50.1	45	4262	9559	9539	50.6	42.9	0.5	420	75.3	41.4	36	52.8
743	1763	12235	12253	50.1	52.6	4263	12998	12979	50.1	45	0	764	76.5	42.5	36	53.7
744	1764	3225	3244	52.4	55	4264	3503	3484	51.5	50	1	279	74.8	42.3	36	52.9
745	1765	14951	14975	52.2	40	4265	15595	15576	50.8	45	1.3	645	75.5	40.6	36	53.2
746	1766	3225	3244	52.4	55	4266	3500	3481	51.2	50	1.3	276	74.7	42	36	52.7
747	1767	12233	12251	51.1	52.6	4267	12999	12980	50.6	40	0.6	767	76.4	42.5	36	53.8
748	1768	3225	3244	52.4	55	4268	3497	3478	51.3	50	1.1	273	74.7	42.1	36	52.8
749	1769	12233	12251	51.1	52.6	4269	13000	12981	51.1	45	0.1	768	76.5	42.6	36	54
750	1770	28395	28414	51.5	45	4270	28671	28653	50.2	52.6	1.3	277	78.5	51.3	36	55.1
751	1771	28395	28414	51.5	45	4271	28671	28652	52.8	55	1.3	277	78.5	51.3	36	55.5
752	1772	9931	9950	50.2	45	4272	10449	10431	50.9	47.4	0.8	519	75.3	40.8	36	52.9
753	1773	12235	12253	50.1	52.6	4273	12994	12976	50.3	47.4	0.2	760	76.4	42.5	36	53.6
754	1774	3359	3379	51.2	42.9	4274	3650	3631	53.1	50	1.9	292	75.6	43.8	36	53.4
755	1775	11543	11562	50.4	40	4275	12258	12238	50.3	42.9	0.2	716	76.1	41.9	36	53.5
756	1776	28396	28416	52.4	47.6	4276	28672	28653	51.8	55	0.5	277	78.6	51.6	36	55.7
757	1777	28396	28416	52.4	47.6	4277	28671	28652	52.8	55	0.4	276	78.6	51.4	36	55.8
758	1778	3229	3248	50.6	50	4278	3647	3628	50.6	45	0	419	75.3	41.5	36	53
759	1779	12235	12253	50.1	52.6	4279	12992	12974	51.2	52.6	1.1	758	76.5	42.6	36	53.7
760	1780	3229	3248	50.6	50	4280	3646	3625	52	40.9	1.4	418	75.3	41.4	36	53
761	1781	3228	3248	52	47.6	4281	3650	3631	53.1	50	1.1	423	75.4	41.6	36	53.5
762	1782	3230	3249	50.1	45	4282	3647	3628	50.6	45	0.5	418	75.3	41.4	36	52.8
763	1783	9931	9950	50.2	45	4283	10449	10428	51.9	40.9	1.8	519	75.3	40.8	36	52.9
764	1784	1402	1422	50.2	42.9	4284	1622	1602	51.6	47.6	1.4	221	76.5	48	36	53.7
765	1785	9922	9941	51.3	50	4285	10608	10589	51	50	0.3	687	75.8	41.2	36	53.5
766	1786	3792	3810	52.9	52.6	4286	4318	4294	54.4	40	1.5	527	75.5	41.2	36	53.8
767	1787	2429	2447	50.2	47.4	4287	3189	3168	51	45.5	0.7	761	76.6	43	36	53.8
768	1788	18008	18029	54.5	50	4288	18443	18424	55.9	55	1.4	436	76	43.1	36	54.7
769	1789	13039	13058	51.8	50	4289	13179	13158	50.4	40.9	1.5	141	74	46.1	36	52
770	1790	942	961	52.8	50	4290	1484	1466	53.1	52.6	0.4	543	76.9	44.4	36	54.7
771	1791	943	961	50.3	47.4	4291	1483	1464	51.3	45	1	541	76.8	44.2	36	53.9
772	1792	28867	28886	53.2	50	4292	29358	29339	52.8	50	0.3	492	76.9	44.9	36	54.8
773	1793	943	961	50.3	47.4	4293	1483	1465	50.5	47.4	0.3	541	76.8	44.2	36	53.9
774	1794	28866	28886	55.4	52.4	4294	29301	29282	55.3	55	0.2	436	77	45.4	36	55.6
775	1795	12352	12375	52.9	41.7	4295	12997	12977	51.8	42.9	1.1	646	76.4	42.9	36	54.1
776	1796	28867	28887	53.7	47.6	4296	29358	29339	52.8	50	0.9	492	76.9	44.9	36	54.8
777	1797	3896	3917	50.7	40.9	4297	4608	4590	51.5	52.6	0.9	713	75.5	40.4	36	53.2
778	1798	6098	6118	50.3	42.9	4298	6486	6467	50.8	45	0.5	389	74.6	40.1	36	52.4
779	1799	28868	28888	51.4	42.9	4299	29358	29339	52.8	50	1.4	491	76.9	44.8	36	54.3
780	1800	8220	8240	54	47.6	4300	8931	8913	55.5	52.6	1.4	712	75.4	40	36	54.1
781	1801	2220	2239	51.3	45	4301	2672	2653	51.6	50	0.4	453	77	45.3	36	54.4

782	1802	12040	12057	50.6	50	4302	12493	12476	50.7	50	0.1	454	76.3	43.6	36	53.7
783	1803	942	960	52.1	52.6	4303	1483	1464	51.3	45	0.8	542	76.8	44.3	36	54.3
784	1804	28868	28889	52	40.9	4304	29358	29339	52.8	50	0.8	491	76.9	44.8	36	54.5
785	1805	942	960	52.1	52.6	4305	1483	1465	50.5	47.4	1.6	542	76.8	44.3	36	54
786	1806	12040	12057	50.6	50	4306	12724	12705	52.4	55	1.8	685	76.6	43.1	36	53.9
787	1807	942	960	52.1	52.6	4307	1484	1466	53.1	52.6	1	543	76.9	44.4	36	54.5
788	1808	11545	11563	50.8	47.4	4308	12253	12235	50.1	52.6	0.7	709	76.2	42.2	36	53.5
789	1809	98	118	50.6	42.9	4309	269	251	51.1	52.6	0.5	172	75	46.5	36	52.8
790	1810	12373	12391	50.8	47.4	4310	12911	12892	50.5	50	0.3	539	76.1	42.5	36	53.5
791	1811	16366	16384	50.3	52.6	4311	16781	16761	51.3	47.6	0.9	416	75.1	41.1	36	52.8
792	1812	9929	9946	50	50	4312	10183	10165	51.7	47.4	1.6	255	75.3	43.9	36	52.8
793	1813	12236	12256	51.2	42.9	4313	13000	12981	51.1	45	0.1	765	76.4	42.5	36	53.9
794	1814	3231	3252	52.7	45.5	4314	3650	3631	53.1	50	0.4	420	75.4	41.7	36	53.7
795	1815	11541	11560	50.1	45	4315	11727	11708	50.4	45	0.3	187	73	40.6	36	51.2
796	1816	3232	3252	51.1	47.6	4316	3494	3473	50.4	40.9	0.7	263	74.3	41.4	36	52.3
797	1817	7725	7743	50.8	47.4	4317	8054	8035	50.4	50	0.4	330	75	41.8	36	52.7
798	1818	28968	28988	50.9	47.6	4318	29358	29339	52.8	50	2	391	76.4	44.5	36	53.9
799	1819	11545	11563	50.8	47.4	4319	12257	12237	51.3	47.6	0.5	713	76.2	42.1	36	53.7
800	1820	24417	24436	52.6	50	4320	25080	25062	53.5	52.6	0.9	664	75.8	41.3	36	53.9
801	1821	28968	28989	51.5	45.5	4321	29358	29339	52.8	50	1.3	391	76.4	44.5	36	54.1
802	1822	3789	3808	53.5	50	4322	4318	4294	54.4	40	0.9	530	75.5	41.1	36	54
803	1823	3232	3252	51.1	47.6	4323	3646	3625	52	40.9	0.9	415	75.3	41.4	36	53.1
804	1824	3232	3252	51.1	47.6	4324	3647	3628	50.6	45	0.5	416	75.3	41.6	36	53
805	1825	28971	28993	51.9	43.5	4325	29306	29288	53.5	52.6	1.6	336	76.2	44.6	36	54
806	1826	24179	24200	53.3	40.9	4326	24818	24797	51.6	40.9	1.6	640	75.8	41.2	36	53.6
807	1827	3231	3251	52	47.6	4327	3650	3631	53.1	50	1.1	420	75.4	41.7	36	53.5
808	1828	9930	9950	52.6	47.6	4328	10449	10425	54.6	40	2	520	75.4	41	36	53.7
809	1829	8866	8885	51.1	45	4329	9254	9236	50.6	47.4	0.5	389	75	41.1	36	52.8
810	1830	2522	2541	51.4	45	4330	2672	2653	51.6	50	0.2	151	75.3	48.3	36	53.2
811	1831	11541	11561	50.9	42.9	4331	12258	12238	50.3	42.9	0.6	718	76.2	41.9	36	53.5
812	1832	3232	3251	50.3	50	4332	3646	3625	52	40.9	1.7	415	75.3	41.4	36	52.9
813	1833	3232	3251	50.3	50	4333	3647	3628	50.6	45	0.3	416	75.3	41.6	36	52.9
814	1834	23843	23863	50.3	42.9	4334	24527	24508	50.5	45	0.2	685	76	41.8	36	53.4
815	1835	21210	21228	53.2	52.6	4335	21317	21293	53.2	40	0	108	71.1	42.6	36	50.8
816	1836	3229	3249	51.4	47.6	4336	3650	3631	53.1	50	1.7	422	75.4	41.7	36	53.3
817	1837	3230	3249	50.1	45	4337	3494	3473	50.4	40.9	0.3	265	74.2	41.1	36	52.1
818	1838	2371	2389	50.3	47.4	4338	2997	2976	51.4	40.9	1.1	627	76.7	43.5	36	53.9
819	1839	29186	29206	51.3	42.9	4339	29298	29280	51.4	52.6	0.1	113	72.8	46	36	51.5
820	1840	9929	9946	50	50	4340	10455	10435	50.5	42.9	0.4	527	75.3	40.6	36	52.8
821	1841	9351	9370	51.2	50	4341	9989	9968	51	40.9	0.2	639	75.4	40.4	36	53.2
822	1842	25348	25365	50.4	50	4342	25772	25753	51.9	50	1.5	425	74.9	40.5	36	52.6
823	1843	1402	1422	50.2	42.9	4343	2103	2082	52	45.5	1.8	702	76.7	43.3	36	53.8
824	1844	9929	9946	50	50	4344	10608	10589	51	50	0.9	680	75.8	41.2	36	53.2
825	1845	9934	9953	50.7	50	4345	10608	10589	51	50	0.3	675	75.8	41.2	36	53.4
826	1846	13176	13196	51.4	47.6	4346	13544	13525	52.6	55	1.2	369	77.1	46.3	36	54.5
827	1847	7725	7743	50.8	47.4	4347	8189	8170	50.6	50	0.2	465	75.6	41.9	36	53.2
828	1848	7725	7743	50.8	47.4	4348	8190	8172	50.3	47.4	0.6	466	75.6	41.8	36	53.1
829	1849	18074	18093	50.3	45	4349	18662	18641	50.4	40.9	0.1	589	76.2	42.6	36	53.6
830	1850	18074	18093	50.3	45	4350	18632	18611	50.2	40.9	0.1	559	76.2	42.8	36	53.5
831	1851	29200	29222	53.2	43.5	4351	29306	29288	53.5	52.6	0.3	107	73.1	47.7	36	52.3
832	1852	25348	25366	51.2	47.4	4352	25545	25526	51.7	45	0.5	198	74.1	42.9	36	52.3

833	1853	25348	25366	51.2	47.4	4353	25545	25525	52.3	42.9	1.1	198	74.1	42.9	36	52.3
834	1854	29200	29223	53.7	41.7	4354	29306	29288	53.5	52.6	0.2	107	73.1	47.7	36	52.3
835	1855	25347	25366	52.7	50	4355	25545	25521	54.5	40	1.8	199	74.2	43.2	36	52.9
836	1856	3792	3811	54	55	4356	4447	4425	53	43.5	1	656	75.5	40.5	36	53.9
837	1857	29200	29224	54.2	40	4357	29306	29288	53.5	52.6	0.7	107	73.1	47.7	36	52.3
838	1858	985	1004	51.1	50	4358	1483	1465	50.5	47.4	0.6	499	76.4	43.5	36	53.7
839	1859	2427	2445	52.1	52.6	4359	3189	3168	51	45.5	1.1	763	76.7	43.1	36	54.1
840	1860	13701	13725	53.6	40	4360	14084	14060	53.6	40	0.1	384	74.6	40.1	36	53.4
841	1861	985	1004	51.1	50	4361	1483	1464	51.3	45	0.2	499	76.4	43.5	36	53.9
842	1862	8794	8813	51.6	45	4362	9559	9539	50.6	42.9	1	766	75.9	41.3	37	53.4
843	1863	3789	3806	50	50	4363	4435	4417	50.5	52.6	0.4	647	75.5	40.5	37	52.9
844	1864	13177	13197	50.3	42.9	4364	13314	13297	51	50	0.6	138	72.8	43.5	37	51.2
845	1865	3791	3808	50	50	4365	4435	4417	50.5	52.6	0.4	645	75.4	40.5	37	52.9
846	1866	9139	9159	52.5	47.6	4366	9364	9346	53.9	52.6	1.4	226	74.9	43.8	37	53.3
847	1867	3226	3245	51.7	55	4367	3494	3473	50.4	40.9	1.3	269	74.5	41.6	37	52.4
848	1868	13040	13059	50.9	50	4368	13314	13297	51	50	0.1	275	75.6	44.4	37	53.3
849	1869	2522	2541	51.4	45	4369	2891	2873	50.8	47.4	0.6	370	76	43.8	37	53.6
850	1870	8865	8884	50.4	45	4370	9245	9226	50	45	0.4	381	74.9	40.9	37	52.6
851	1871	3787	3804	50	50	4371	4434	4416	51.5	52.6	1.4	648	75.4	40.3	37	52.9
852	1872	3226	3245	51.7	55	4372	3646	3625	52	40.9	0.3	421	75.3	41.6	37	53.4
853	1873	3226	3245	51.7	55	4373	3647	3628	50.6	45	1.1	422	75.4	41.7	37	53.1
854	1874	3226	3245	51.7	55	4374	3650	3631	53.1	50	1.4	425	75.5	41.9	37	53.5
855	1875	2387	2405	51.6	52.6	4375	2747	2727	50	42.9	1.6	361	76.9	46	37	53.9
856	1876	18074	18093	50.3	45	4376	18229	18209	50.1	42.9	0.2	156	73.2	42.9	37	51.4
857	1877	13701	13725	53.6	40	4377	14059	14040	52.8	50	0.8	359	74.5	40.1	37	53.1
858	1878	3787	3804	50	50	4378	4435	4417	50.5	52.6	0.4	649	75.4	40.4	37	52.9
859	1879	13040	13059	50.9	50	4379	13323	13304	51.1	45	0.2	284	75.7	44.4	37	53.4
860	1880	3789	3806	50	50	4380	4434	4416	51.5	52.6	1.4	646	75.4	40.4	37	52.9
861	1881	15506	15527	50.8	40.9	4381	16214	16196	51.8	52.6	1	709	75.5	40.3	37	53.2
862	1882	12234	12252	50.6	47.4	4382	12412	12392	50	42.9	0.6	179	73.1	41.3	37	51.3
863	1883	12234	12252	50.6	47.4	4383	12739	12718	51	40.9	0.4	506	75.8	42.1	37	53.4
864	1884	18074	18094	51.1	42.9	4384	18229	18209	50.1	42.9	0.9	156	73.2	42.9	37	51.4
865	1885	18075	18095	50.6	47.6	4385	18223	18206	51.8	50	1.2	149	73.3	43.6	37	51.6
866	1886	13040	13059	50.9	50	4386	13326	13306	50.7	42.9	0.2	287	75.7	44.3	37	53.3
867	1887	18080	18098	51.2	52.6	4387	18233	18215	51.3	52.6	0.1	154	73.9	44.8	37	52.2
868	1888	18080	18098	51.2	52.6	4388	18233	18214	52	50	0.9	154	73.9	44.8	37	52.2
869	1889	18080	18098	51.2	52.6	4389	18231	18210	52.2	45.5	1	152	73.5	44.1	37	51.9
870	1890	18080	18098	51.2	52.6	4390	18223	18206	51.8	50	0.6	144	73.2	43.8	37	51.7
871	1891	18077	18098	52.9	50	4391	18220	18202	54.8	52.6	1.9	144	73.2	43.8	37	52.2
872	1892	18076	18098	54.4	47.8	4392	18443	18424	55.9	55	1.5	368	75.8	43.2	37	54.5
873	1893	3792	3810	52.9	52.6	4393	4436	4417	52.2	50	0.6	645	75.4	40.5	37	53.6
874	1894	3055	3074	51.1	50	4394	3647	3628	50.6	45	0.5	593	76.3	42.7	37	53.7
875	1895	3055	3074	51.1	50	4395	3646	3625	52	40.9	0.9	592	76.2	42.6	37	53.8
876	1896	15506	15527	50.8	40.9	4396	15645	15625	51.1	42.9	0.4	140	71.8	40.7	37	50.6
877	1897	18081	18099	51.2	52.6	4397	18229	18209	50.1	42.9	1.1	149	73.3	43.6	37	51.4
878	1898	13039	13058	51.8	50	4398	13155	13138	50.4	50	1.4	117	73.4	47	37	51.6
879	1899	3055	3075	51.8	47.6	4399	3650	3631	53.1	50	1.3	596	76.3	42.8	37	54.1
880	1900	18080	18099	53	50	4400	18223	18205	53.3	52.6	0.4	144	73.2	43.8	37	52.2
881	1901	27361	27380	52.4	55	4401	27579	27558	51.1	40.9	1.3	219	75.4	45.2	37	53.2
882	1902	3221	3239	51.5	52.6	4402	3503	3484	51.5	50	0	283	74.8	42	37	52.9
883	1903	3221	3239	51.5	52.6	4403	3504	3485	50.4	45	1.1	284	74.7	41.9	37	52.5

884	1904	18077	18099	54.4	47.8	4404	18220	18201	56.1	55	1.7	144	73.2	43.8	37	52.6
885	1905	3055	3075	51.8	47.6	4405	3647	3628	50.6	45	1.2	593	76.3	42.7	37	53.7
886	1906	18581	18599	51.4	47.4	4406	18697	18679	51.9	52.6	0.4	117	71	41	37	50.2
887	1907	18616	18636	51.4	47.6	4407	19216	19195	50.2	40.9	1.1	601	75.6	41.1	37	53.1
888	1908	3219	3238	50.7	50	4408	3503	3484	51.5	50	0.8	285	74.8	42.1	37	52.7
889	1909	18696	18715	51.7	50	4409	19216	19195	50.2	40.9	1.5	521	75.5	41.3	37	53
890	1910	3219	3238	50.7	50	4410	3504	3485	50.4	45	0.3	286	74.7	42	37	52.5
891	1911	27366	27384	52.2	52.6	4411	27573	27552	52.3	40.9	0.1	208	74.6	43.8	37	53
892	1912	27366	27384	52.2	52.6	4412	27567	27547	51.1	42.9	1	202	74.6	44.1	37	52.7
893	1913	3055	3075	51.8	47.6	4413	3646	3625	52	40.9	0.2	592	76.2	42.6	37	54
894	1914	18704	18724	50.8	47.6	4414	19216	19195	50.2	40.9	0.5	513	75.5	41.1	37	53
895	1915	16874	16893	52.1	50	4415	17056	17035	51.8	45.5	0.4	183	74.4	44.3	37	52.7
896	1916	12234	12252	50.6	47.4	4416	12739	12719	50.3	42.9	0.3	506	75.8	42.1	37	53.3
897	1917	7728	7746	51.7	52.6	4417	8054	8035	50.4	50	1.2	327	75	41.9	37	52.7
898	1918	15506	15527	50.8	40.9	4418	15647	15628	51	45	0.3	142	71.9	40.8	37	50.7
899	1919	985	1004	51.1	50	4419	1773	1755	51.7	52.6	0.6	789	76.7	43.1	37	54.1
900	1920	3217	3236	51.1	50	4420	3503	3484	51.5	50	0.4	287	74.8	42.2	37	52.8
901	1921	3791	3808	50	50	4421	4434	4416	51.5	52.6	1.4	644	75.4	40.4	37	52.9
902	1922	19794	19813	50	50	4422	19923	19904	50.1	50	0.1	130	72.7	43.8	37	51
903	1923	13039	13058	51.8	50	4423	13178	13157	50.4	40.9	1.5	140	73.8	45.7	37	51.9
904	1924	13033	13051	52.1	52.6	4424	13155	13138	50.4	50	1.7	123	73.7	47.2	37	51.8
905	1925	12233	12251	51.1	52.6	4425	12739	12719	50.3	42.9	0.9	507	75.9	42.2	37	53.3
906	1926	19795	19814	50.4	45	4426	19923	19903	50.9	47.6	0.4	129	72.5	43.4	37	51
907	1927	13177	13197	50.3	42.9	4427	13946	13929	51.5	50	1.2	770	75.9	41	37	53.3
908	1928	3799	3820	52.9	45.5	4428	4318	4294	54.4	40	1.5	520	75.4	41	37	53.7
909	1929	8867	8887	52.3	47.6	4429	9364	9346	53.9	52.6	1.6	498	75.8	42.2	37	53.9
910	1930	1472	1491	51.2	45	4430	2152	2133	50.7	45	0.5	681	76.5	42.9	37	53.8
911	1931	12233	12251	51.1	52.6	4431	12739	12718	51	40.9	0.2	507	75.9	42.2	37	53.5
912	1932	3055	3076	52.4	45.5	4432	3650	3631	53.1	50	0.7	596	76.3	42.8	37	54.2
913	1933	12726	12746	51.3	47.6	4433	13325	13305	50.5	47.6	0.7	600	76.7	43.7	37	53.9
914	1934	8867	8887	52.3	47.6	4434	9316	9296	50.8	42.9	1.5	450	75.4	41.6	37	53.1
915	1935	8867	8887	52.3	47.6	4435	9314	9295	51.1	50	1.2	448	75.5	41.7	37	53.3
916	1936	8867	8887	52.3	47.6	4436	9313	9294	50.4	50	1.9	447	75.5	41.6	37	53
917	1937	3055	3076	52.4	45.5	4437	3647	3628	50.6	45	1.8	593	76.3	42.7	37	53.7
918	1938	13176	13196	51.4	47.6	4438	13312	13294	51	52.6	0.4	137	72.9	43.8	37	51.5
919	1939	12726	12746	51.3	47.6	4439	13155	13138	50.4	50	0.9	430	76.4	44	37	53.7
920	1940	13701	13724	53.1	41.7	4440	14058	14040	51.4	52.6	1.7	358	74.5	40.2	37	52.7
921	1941	8372	8390	50.7	47.4	4441	9101	9081	50.5	47.6	0.2	730	75.5	40.3	37	53.1
922	1942	3055	3076	52.4	45.5	4442	3646	3625	52	40.9	0.4	592	76.2	42.6	37	54.1
923	1943	887	905	50.1	47.4	4443	1493	1474	50.8	45	0.7	607	77.1	44.6	37	54.1
924	1944	1046	1063	50.3	50	4444	1697	1677	51	42.9	0.7	652	76.9	43.9	37	54
925	1945	27378	27397	50.5	45	4445	27675	27656	50	40	0.5	298	74.1	40.3	37	52
926	1946	27378	27397	50.5	45	4446	27674	27654	51.9	42.9	1.4	297	74.2	40.4	37	52.2
927	1947	2671	2692	52.1	40.9	4447	3056	3037	52.1	55	0	386	74.8	40.7	37	53.1
928	1948	1046	1063	50.3	50	4448	1697	1678	50.3	45	0.1	652	76.9	43.9	37	54
929	1949	2387	2405	51.6	52.6	4449	2672	2654	50.9	52.6	0.8	286	77	47.6	37	54.3
930	1950	3792	3810	52.9	52.6	4450	4565	4542	53.9	41.7	1	774	75.6	40.3	37	53.8
931	1951	15506	15527	50.8	40.9	4451	15647	15629	50.3	47.4	0.5	142	71.9	40.8	37	50.5
932	1952	8794	8813	51.6	45	4452	9560	9540	51.6	42.9	0	767	75.9	41.2	37	53.7
933	1953	19801	19819	53.2	52.6	4453	19909	19885	52.5	40	0.7	109	71.4	43.1	37	50.8
934	1954	19988	20006	50.4	47.4	4454	20615	20597	50.6	47.4	0.2	628	75.3	40.1	37	52.9

935	1955	19991	20009	52.8	52.6	4455	20616	20597	52.3	45	0.5	626	75.3	40.1	37	53.5
936	1956	16875	16895	51.6	47.6	4456	17060	17041	51.1	50	0.5	186	74.6	44.6	37	52.6
937	1957	16875	16895	51.6	47.6	4457	17059	17039	50.6	47.6	1	185	74.4	44.3	37	52.4
938	1958	16875	16895	51.6	47.6	4458	17056	17035	51.8	45.5	0.2	182	74.2	44	37	52.5
939	1959	27442	27461	51.5	40	4459	27541	27521	51.7	47.6	0.1	100	71.2	44	37	50.4
940	1960	16875	16896	52.2	45.5	4460	17060	17041	51.1	50	1.1	186	74.6	44.6	37	52.6
941	1961	23841	23859	50.5	52.6	4461	24527	24507	51	42.9	0.5	687	76.1	41.9	37	53.5
942	1962	23841	23859	50.5	52.6	4462	24093	24075	50.9	52.6	0.4	253	76	45.8	37	53.5
943	1963	16875	16896	52.2	45.5	4463	17059	17039	50.6	47.6	1.6	185	74.4	44.3	37	52.4
944	1964	16875	16896	52.2	45.5	4464	17056	17035	51.8	45.5	0.5	182	74.2	44	37	52.6
945	1965	23843	23863	50.3	42.9	4465	24093	24075	50.9	52.6	0.5	251	75.8	45.4	37	53.3
946	1966	16875	16896	52.2	45.5	4466	17041	17023	53.5	52.6	1.3	167	73.8	43.7	37	52.4
947	1967	28187	28205	53.1	52.6	4467	28673	28654	53.5	55	0.5	487	80	52.4	37	57
948	1968	28190	28208	51.7	52.6	4468	28672	28654	50.6	52.6	1.2	483	79.9	52.2	37	56.2
949	1969	24030	24047	50.7	50	4469	24527	24508	50.5	45	0.2	498	75.4	41.2	37	53
950	1970	24031	24050	56.5	55	4470	24816	24792	54.7	40	1.8	786	76.3	42	37	54.9
951	1971	7880	7900	50.3	42.9	4471	8049	8032	50.4	50	0	170	72.8	41.2	37	51.2
952	1972	24096	24119	54.4	41.7	4472	24815	24791	54.5	40	0.1	720	75.8	41	37	54.5
953	1973	17790	17811	51.6	40.9	4473	18233	18214	52	50	0.4	444	75.1	40.8	37	53.2
954	1974	24174	24194	50.9	42.9	4474	24938	24921	50.4	50	0.5	765	75.8	40.9	37	53.3
955	1975	16875	16896	52.2	45.5	4475	17039	17022	51.4	50	0.8	165	73.7	43.6	37	52.1
956	1976	24174	24195	52.5	40.9	4476	24936	24919	51.8	50	0.7	763	75.8	41	37	53.7
957	1977	24179	24198	51	45	4477	24938	24921	50.4	50	0.6	760	75.8	40.9	37	53.3
958	1978	16875	16896	52.2	45.5	4478	17038	17021	50.7	50	1.6	164	73.8	43.9	37	52
959	1979	24180	24199	50.3	40	4479	24936	24919	51.8	50	1.5	757	75.8	41	37	53.2
960	1980	2823	2844	50.4	45.5	4480	3186	3165	50.4	40.9	0	364	75.5	42.6	37	53.1
961	1981	10142	10163	51.3	40.9	4481	10608	10589	51	50	0.3	467	74.9	40	37	52.8
962	1982	1046	1063	50.3	50	4482	1483	1464	51.3	45	0.9	438	76.2	43.6	37	53.6
963	1983	17388	17408	50.7	42.9	4483	17501	17481	51.2	42.9	0.6	114	70.5	40.4	37	49.7
964	1984	24179	24200	53.3	40.9	4484	24740	24717	52.5	41.7	0.8	562	76	42.2	37	54
965	1985	24379	24398	55	55	4485	25088	25070	54.5	52.6	0.5	710	75.9	41.3	37	54.6
966	1986	24379	24398	55	55	4486	25087	25069	53.7	52.6	1.3	709	75.9	41.3	37	54.3
967	1987	16874	16893	52.1	50	4487	17059	17039	50.6	47.6	1.5	186	74.6	44.6	37	52.5
968	1988	24380	24399	55	55	4488	25088	25070	54.5	52.6	0.5	709	75.9	41.3	37	54.6
969	1989	28522	28542	50.2	42.9	4489	28671	28653	50.2	52.6	0	150	76.2	50.7	37	53.5
970	1990	16874	16893	52.1	50	4490	17060	17041	51.1	50	1	187	74.7	44.9	37	52.7
971	1991	24380	24399	55	55	4491	25087	25069	53.7	52.6	1.3	708	75.9	41.4	37	54.4
972	1992	17608	17627	50.2	45	4492	18239	18220	50	45	0.2	632	75.3	40.2	37	52.8
973	1993	17608	17627	50.2	45	4493	18238	18219	50.3	45	0.1	631	75.3	40.3	37	52.9
974	1994	1046	1063	50.3	50	4494	1483	1465	50.5	47.4	0.2	438	76.2	43.6	37	53.6
975	1995	17608	17628	50.9	42.9	4495	18239	18220	50	45	0.9	632	75.3	40.2	37	52.8
976	1996	17608	17628	50.9	42.9	4496	18238	18219	50.3	45	0.7	631	75.3	40.3	37	52.9
977	1997	8063	8084	51.4	45.5	4497	8188	8169	50.5	45	0.9	126	72.1	42.9	37	50.7
978	1998	12236	12256	51.2	42.9	4498	12739	12718	51	40.9	0.2	504	75.8	42.1	37	53.5
979	1999	13176	13196	51.4	47.6	4499	13325	13305	50.5	47.6	0.8	150	73.4	44	37	51.7
980	2000	2371	2389	50.3	47.4	4500	2749	2728	50.3	45.5	0	379	76.9	45.9	37	54.1
981	2001	9402	9420	51.3	47.4	4501	9989	9968	51	40.9	0.4	588	75.4	40.5	37	53.1
982	2002	9931	9950	50.2	45	4502	10183	10166	50.9	50	0.7	253	75.2	43.9	37	52.8
983	2003	2387	2405	51.6	52.6	4503	2997	2976	51.4	40.9	0.2	611	76.6	43.5	37	54.2
984	2004	3788	3805	50	50	4504	4435	4417	50.5	52.6	0.4	648	75.4	40.4	37	52.9
985	2005	26039	26057	52.6	52.6	4505	26650	26630	51.4	42.9	1.2	612	75.3	40.4	37	53.3

986	2006	2371	2389	50.3	47.4	4506	3053	3034	50.3	50	0	683	76.7	43.3	37	53.9
987	2007	3	21	53.4	52.6	4507	315	296	51.9	50	1.5	313	76.9	46.6	37	54.5
988	2008	2371	2389	50.3	47.4	4508	3056	3037	52.1	55	1.7	686	76.7	43.4	37	53.9
989	2009	13040	13059	50.9	50	4509	13155	13137	52.1	52.6	1.2	116	73.2	46.6	37	51.6
990	2010	9931	9950	50.2	45	4510	10183	10165	51.7	47.4	1.5	253	75.2	43.9	37	52.8
991	2011	3788	3805	50	50	4511	4434	4416	51.5	52.6	1.4	647	75.4	40.3	37	52.9
992	2012	13176	13196	51.4	47.6	4512	13946	13929	51.5	50	0.2	771	75.9	41.1	37	53.6
993	2013	3772	3792	51.2	42.9	4513	4444	4424	50.6	42.9	0.7	673	75.6	40.7	37	53.2
994	2014	13176	13196	51.4	47.6	4514	13320	13300	51.4	47.6	0	145	73.3	44.1	37	51.9
995	2015	8861	8880	50.2	45	4515	9245	9226	50	45	0.1	385	74.9	40.8	37	52.5
996	2016	8868	8889	50.4	40.9	4516	9310	9291	51.2	45	0.8	443	75.3	41.3	37	52.9
997	2017	16366	16384	50.3	52.6	4517	16774	16752	52.2	43.5	1.9	409	75.1	41.1	37	52.8
998	2018	9934	9953	50.7	50	4518	10183	10166	50.9	50	0.2	250	75.2	44	37	53
999	2019	9055	9079	52.8	40	4519	9342	9323	52.1	50	0.8	288	75.1	42.7	37	53.3
1000	2020	8868	8889	50.4	40.9	4520	9249	9231	50.8	47.4	0.4	382	75.1	41.4	37	52.8
1001	2021	16366	16385	52.9	55	4521	16774	16752	52.2	43.5	0.6	409	75.1	41.1	37	53.3
1002	2022	8868	8889	50.4	40.9	4522	9249	9230	51.5	45	1.1	382	75.1	41.4	37	52.8
1003	2023	9934	9953	50.7	50	4523	10183	10165	51.7	47.4	0.9	250	75.2	44	37	53
1004	2024	25772	25793	52.4	40.9	4524	26183	26163	51.7	42.9	0.7	412	74.8	40.3	37	53
1005	2025	13039	13057	51.1	52.6	4525	13155	13138	50.4	50	0.7	117	73.4	47	37	51.6
1006	2026	25771	25790	51.1	45	4526	26183	26164	51	45	0.1	413	74.8	40.4	37	52.8
1007	2027	25769	25786	50.3	50	4527	26183	26163	51.7	42.9	1.4	415	74.9	40.5	37	52.6
1008	2028	3794	3812	52.9	52.6	4528	4436	4417	52.2	50	0.6	643	75.4	40.4	37	53.6
1009	2029	887	905	50.1	47.4	4529	1480	1462	51.6	47.4	1.5	594	77.1	44.6	37	54.1
1010	2030	3794	3812	52.9	52.6	4530	4434	4416	51.5	52.6	1.4	641	75.4	40.4	37	53.3
1011	2031	12370	12388	50.1	47.4	4531	12994	12976	50.3	47.4	0.3	625	76.4	42.9	37	53.6
1012	2032	3797	3815	50.9	47.4	4532	4186	4168	51.8	52.6	0.9	390	75.3	41.8	37	53.1
1013	2033	3795	3813	52.1	52.6	4533	4435	4417	50.5	52.6	1.6	641	75.5	40.6	37	53.1
1014	2034	3795	3813	52.1	52.6	4534	4434	4416	51.5	52.6	0.6	640	75.4	40.5	37	53.3
1015	2035	13177	13197	50.3	42.9	4535	13323	13304	51.1	45	0.8	147	73.2	43.5	37	51.4
1016	2036	1046	1064	51.2	47.4	4536	1401	1382	50.6	45	0.6	356	75.6	43	37	53.2
1017	2037	16549	16567	54.9	52.6	4537	17057	17035	53	43.5	1.9	509	75.9	42.2	37	54.1
1018	2038	1046	1064	51.2	47.4	4538	1483	1464	51.3	45	0.1	438	76.2	43.6	37	53.8
1019	2039	16551	16568	51.1	50	4539	17056	17035	51.8	45.5	0.7	506	75.9	42.3	37	53.6
1020	2040	1046	1064	51.2	47.4	4540	1483	1465	50.5	47.4	0.6	438	76.2	43.6	37	53.6
1021	2041	1046	1064	51.2	47.4	4541	1484	1466	53.1	52.6	2	439	76.3	43.7	37	53.9
1022	2042	1046	1064	51.2	47.4	4542	1697	1676	51.7	40.9	0.5	652	76.9	43.9	37	54.2
1023	2043	1046	1064	51.2	47.4	4543	1697	1677	51	42.9	0.2	652	76.9	43.9	37	54.2
1024	2044	16555	16572	50.3	50	4544	17111	17090	51.1	40.9	0.8	557	76.1	42.5	37	53.5
1025	2045	1046	1064	51.2	47.4	4545	1697	1678	50.3	45	0.9	652	76.9	43.9	37	54
1026	2046	1046	1063	50.3	50	4546	1401	1382	50.6	45	0.2	356	75.6	43	37	53.1
1027	2047	3796	3814	50.8	52.6	4547	4435	4417	50.5	52.6	0.3	640	75.4	40.5	37	53.1
1028	2048	12232	12250	51.9	52.6	4548	12993	12975	51.4	47.4	0.5	762	76.5	42.5	37	54
1029	2049	12236	12256	51.2	42.9	4549	12739	12719	50.3	42.9	0.9	504	75.8	42.1	37	53.2
1030	2050	28937	28956	52.4	50	4550	29306	29288	53.5	52.6	1.1	370	76.6	45.1	37	54.4
1031	2051	12232	12250	51.9	52.6	4551	12996	12977	50.2	40	1.7	765	76.4	42.4	37	53.6
1032	2052	3234	3254	51.1	47.6	4552	3504	3485	50.4	45	0.7	271	74.4	41.3	37	52.3
1033	2053	3234	3254	51.1	47.6	4553	3503	3484	51.5	50	0.4	270	74.4	41.5	37	52.5
1034	2054	9922	9941	51.3	50	4554	10670	10649	51.3	40.9	0.1	749	75.8	40.9	37	53.5
1035	2055	3792	3810	52.9	52.6	4555	4434	4416	51.5	52.6	1.4	643	75.4	40.4	37	53.3
1036	2056	3234	3254	51.1	47.6	4556	3494	3473	50.4	40.9	0.6	261	74.1	41	37	52.1

1037	2057	4255	4276	51.7	45.5	4557	4608	4590	51.5	52.6	0.2	354	74.7	40.7	37	52.8
1038	2058	24562	24580	50.1	52.6	4558	24936	24919	51.8	50	1.7	375	75.6	42.7	37	53
1039	2059	24562	24580	50.1	52.6	4559	24938	24921	50.4	50	0.3	377	75.5	42.4	37	53
1040	2060	24562	24580	50.1	52.6	4560	25182	25164	51.4	47.4	1.3	621	75.9	41.7	37	53.3
1041	2061	24559	24579	52	52.4	4561	24936	24919	51.8	50	0.2	378	75.7	42.9	37	53.6
1042	2062	24559	24579	52	52.4	4562	24938	24921	50.4	50	1.6	380	75.6	42.6	37	53.1
1043	2063	1046	1063	50.3	50	4563	1697	1676	51.7	40.9	1.3	652	76.9	43.9	37	54
1044	2064	24482	24503	51.6	40.9	4564	24815	24792	53.4	41.7	1.8	334	75.4	42.8	37	53.4
1045	2065	13177	13197	50.3	42.9	4565	13326	13306	50.7	42.9	0.4	150	73.2	43.3	37	51.4
1046	2066	24480	24502	54.2	47.8	4566	24815	24791	54.5	40	0.3	336	75.6	43.2	37	54.3
1047	2067	17840	17859	50.8	45	4567	18223	18206	51.8	50	1	384	74.7	40.4	37	52.6
1048	2068	24480	24500	53.2	47.6	4568	24815	24792	53.4	41.7	0.2	336	75.6	43.2	37	54
1049	2069	17840	17859	50.8	45	4569	18231	18210	52.2	45.5	1.4	392	74.8	40.6	37	52.7
1050	2070	28821	28840	51.8	45	4570	29298	29279	52.6	55	0.8	478	77	45.2	37	54.6
1051	2071	24418	24440	55	47.8	4571	24517	24494	53.2	41.7	1.8	100	70.8	43	37	50.6
1052	2072	13701	13722	50.4	40.9	4572	14058	14040	51.4	52.6	1	358	74.5	40.2	37	52.4
1053	2073	28821	28840	51.8	45	4573	29358	29339	52.8	50	1	538	77.1	45	37	54.6
1054	2074	17792	17813	51.6	40.9	4574	18233	18214	52	50	0.4	442	75.1	40.7	37	53.1
1055	2075	24420	24440	50.8	42.9	4575	25079	25061	52.7	52.6	1.9	660	75.7	41.1	37	53.3
1056	2076	28821	28839	51.1	47.4	4576	29298	29279	52.6	55	1.5	478	77	45.2	37	54.3
1057	2077	3796	3814	50.8	52.6	4577	4434	4416	51.5	52.6	0.7	639	75.4	40.4	37	53.1
1058	2078	28821	28839	51.1	47.4	4578	29358	29339	52.8	50	1.7	538	77.1	45	37	54.4
1059	2079	28820	28838	53.7	52.6	4579	29298	29279	52.6	55	1.1	479	77.1	45.3	37	54.8
1060	2080	24418	24439	52.9	45.5	4580	25079	25061	52.7	52.6	0.2	662	75.8	41.2	37	54
1061	2081	28820	28838	53.7	52.6	4581	29358	29339	52.8	50	0.9	539	77.1	45.1	37	54.9
1062	2082	27369	27389	52.5	47.6	4582	27468	27451	51.1	50	1.4	100	71.2	44	38	50.3
1063	2083	7725	7742	50	50	4583	8187	8167	50.4	42.9	0.3	463	75.6	41.9	38	53
1064	2084	16549	16567	54.9	52.6	4584	17040	17021	53.4	50	1.5	492	75.9	42.3	38	54.2
1065	2085	3221	3239	51.5	52.6	4585	3500	3481	51.2	50	0.3	280	74.6	41.8	38	52.7
1066	2086	16549	16567	54.9	52.6	4586	17041	17022	54.1	50	0.8	493	75.8	42.2	38	54.4
1067	2087	20138	20158	50.1	42.9	4587	20615	20597	50.6	47.4	0.5	478	75	40.4	38	52.7
1068	2088	20078	20099	50.5	40.9	4588	20615	20597	50.6	47.4	0.1	538	75.3	40.5	38	52.9
1069	2089	13039	13057	51.1	52.6	4589	13325	13305	50.5	47.6	0.6	287	75.8	44.6	38	53.3
1070	2090	16549	16567	54.9	52.6	4590	17041	17023	53.5	52.6	1.4	493	75.8	42.2	38	54.2
1071	2091	13701	13725	53.6	40	4591	14124	14106	52.4	52.6	1.2	424	75.1	41	38	53.4
1072	2092	12975	12993	51.4	47.4	4592	13320	13300	51.4	47.6	0	346	76.1	44.2	38	53.8
1073	2093	16548	16566	54.9	52.6	4593	16779	16758	53.5	50	1.4	232	74	41.4	38	52.9
1074	2094	3361	3381	50.5	42.9	4594	3500	3481	51.2	50	0.7	140	74.1	46.4	38	52.1
1075	2095	3361	3381	50.5	42.9	4595	3503	3484	51.5	50	1	143	74.4	46.9	38	52.3
1076	2096	16368	16387	50.2	45	4596	16780	16760	51.4	42.9	1.2	413	74.9	40.7	38	52.6
1077	2097	7725	7742	50	50	4597	8188	8168	50.4	42.9	0.3	464	75.6	41.8	38	53
1078	2098	8867	8887	52.3	47.6	4598	9597	9573	53.4	40	1.1	731	75.9	41.2	38	53.9
1079	2099	2223	2244	51.4	45.5	4599	2672	2654	50.9	52.6	0.5	450	77	45.3	38	54.3
1080	2100	10242	10265	51.2	41.7	4600	10605	10588	51.1	50	0.2	364	74.5	40.1	38	52.6
1081	2101	8867	8888	52.7	45.5	4601	9253	9235	51.6	47.4	1.1	387	75.1	41.3	38	53.2
1082	2102	3361	3381	50.5	42.9	4602	3504	3485	50.4	45	0.1	144	74.3	46.5	38	52.2
1083	2103	98	118	50.6	42.9	4603	314	296	50.6	47.4	0	217	75.9	46.5	38	53.4
1084	2104	12233	12251	51.1	52.6	4604	12498	12480	50	47.4	1.1	266	74.8	42.5	38	52.5
1085	2105	9926	9944	50.5	52.6	4605	10455	10434	51.1	40.9	0.6	530	75.3	40.6	38	52.9
1086	2106	3360	3380	51.4	42.9	4606	3497	3478	51.3	50	0.1	138	74	46.4	38	52.3
1087	2107	9926	9944	50.5	52.6	4607	10455	10435	50.5	42.9	0	530	75.3	40.6	38	52.9

1088	2108	10140	10159	52.4	50	4608	10608	10589	51	50	1.4	469	75	40.3	38	52.9
1089	2109	9931	9950	50.2	45	4609	10455	10435	50.5	42.9	0.3	525	75.3	40.6	38	52.8
1090	2110	3219	3238	50.7	50	4610	3500	3481	51.2	50	0.5	282	74.7	41.8	38	52.6
1091	2111	3219	3238	50.7	50	4611	3497	3478	51.3	50	0.6	279	74.7	41.9	38	52.6
1092	2112	3360	3380	51.4	42.9	4612	3500	3481	51.2	50	0.3	141	74	46.1	38	52.3
1093	2113	3360	3380	51.4	42.9	4613	3503	3484	51.5	50	0	144	74.3	46.5	38	52.5
1094	2114	2223	2244	51.4	45.5	4614	2672	2653	51.6	50	0.2	450	77	45.3	38	54.4
1095	2115	9922	9941	51.3	50	4615	10449	10428	51.9	40.9	0.7	528	75.4	40.9	38	53.3
1096	2116	13039	13057	51.1	52.6	4616	13312	13294	51	52.6	0.1	274	75.7	44.5	38	53.4
1097	2117	15951	15973	52.1	43.5	4617	16174	16154	50.4	42.9	1.7	224	73.5	40.6	38	51.7
1098	2118	13176	13196	51.4	47.6	4618	13545	13526	52.9	55	1.5	370	77	46.2	38	54.4
1099	2119	11541	11562	51.5	40.9	4619	11983	11965	53	52.6	1.5	443	75	40.6	38	53.1
1100	2120	2429	2447	50.2	47.4	4620	3056	3038	50.8	52.6	0.6	628	76.3	42.7	38	53.6
1101	2121	11545	11563	50.8	47.4	4621	12258	12238	50.3	42.9	0.5	714	76.2	42	38	53.5
1102	2122	8868	8889	50.4	40.9	4622	9245	9226	50	45	0.4	378	74.9	41	38	52.6
1103	2123	27361	27380	52.4	55	4623	27466	27448	52.3	52.6	0.1	106	72.5	46.2	38	51.5
1104	2124	8861	8880	50.2	45	4624	9340	9319	50.8	45.5	0.6	480	75.5	41.5	38	53
1105	2125	1784	1802	51.8	52.6	4625	2113	2094	50.1	45	1.7	330	76	44.2	38	53.3
1106	2126	8868	8889	50.4	40.9	4626	9107	9086	51.6	45.5	1.2	240	74	41.2	38	52
1107	2127	19795	19814	50.4	45	4627	20099	20078	50.5	40.9	0	305	74.4	40.7	38	52.3
1108	2128	26708	26731	54.2	41.7	4628	27347	27324	52.3	41.7	1.9	640	75.6	40.8	38	53.7
1109	2129	19794	19813	50	50	4629	19920	19899	50.2	40.9	0.2	127	72.3	43.3	38	50.7
1110	2130	3031	3051	51.3	52.4	4630	3650	3631	53.1	50	1.8	620	76.5	43.1	38	54
1111	2131	3031	3051	51.3	52.4	4631	3647	3628	50.6	45	0.7	617	76.4	42.9	38	53.8
1112	2132	19794	19813	50	50	4632	19922	19902	50	42.9	0	129	72.5	43.4	38	50.8
1113	2133	12236	12256	51.2	42.9	4633	12994	12976	50.3	47.4	0.8	759	76.4	42.4	38	53.7
1114	2134	26708	26731	54.2	41.7	4634	27467	27449	52.8	47.4	1.4	760	76	41.3	38	54.1
1115	2135	19716	19737	52.2	45.5	4635	19922	19901	51.5	45.5	0.7	207	73.5	41.1	38	52
1116	2136	19715	19735	52.5	47.6	4636	19922	19901	51.5	45.5	0.9	208	73.6	41.3	38	52.1
1117	2137	3360	3379	50.7	45	4637	3503	3484	51.5	50	0.7	144	74.3	46.5	38	52.3
1118	2138	9055	9079	52.8	40	4638	9364	9346	53.9	52.6	1.1	310	75.3	42.9	38	53.7
1119	2139	1782	1801	52.7	50	4639	1881	1861	54.5	52.4	1.8	100	72.4	47	38	51.6
1120	2140	26708	26727	50	45	4640	27468	27450	51.9	47.4	1.8	761	75.9	41.3	38	53.3
1121	2141	26708	26727	50	45	4641	27468	27451	51.1	50	1.1	761	75.9	41.3	38	53.3
1122	2142	4593	4613	51.5	47.6	4642	4995	4975	51.7	42.9	0.1	403	76	43.4	38	53.8
1123	2143	19709	19730	51.3	40.9	4643	19930	19911	50.7	50	0.5	222	74	41.9	38	52.1
1124	2144	26421	26441	51.5	42.9	4644	26587	26570	50.2	50	1.3	167	72.3	40.1	38	50.8
1125	2145	18979	19000	51.6	45.5	4645	19217	19195	51.7	43.5	0	239	73.5	40.2	38	52.1
1126	2146	18703	18724	53.5	50	4646	19476	19453	53.5	41.7	0	774	75.6	40.3	38	54
1127	2147	4255	4276	51.7	45.5	4647	4708	4690	50.3	47.4	1.4	454	75.1	40.7	38	52.8
1128	2148	3232	3252	51.1	47.6	4648	3503	3484	51.5	50	0.4	272	74.6	41.9	38	52.6
1129	2149	26421	26441	51.5	42.9	4649	26656	26636	51.3	47.6	0.2	236	74.2	41.9	38	52.5
1130	2150	3232	3252	51.1	47.6	4650	3504	3485	50.4	45	0.7	273	74.6	41.8	38	52.4
1131	2151	26421	26441	51.5	42.9	4651	26660	26641	50.2	50	1.3	240	74.2	41.7	38	52.1
1132	2152	26421	26441	51.5	42.9	4652	26683	26665	52.7	52.6	1.2	263	74.8	42.6	38	52.9
1133	2153	26421	26441	51.5	42.9	4653	26686	26669	50.5	50	0.9	266	74.8	42.5	38	52.6
1134	2154	26421	26441	51.5	42.9	4654	26691	26673	51.3	47.4	0.1	271	74.8	42.4	38	52.9
1135	2155	18704	18724	50.8	47.6	4655	19476	19456	50.5	42.9	0.3	773	75.5	40.2	38	53.1
1136	2156	18704	18724	50.8	47.6	4656	19482	19463	50.1	45	0.7	779	75.5	40.2	38	53
1137	2157	942	960	52.1	52.6	4657	1498	1481	51	50	1.1	557	76.9	44.5	38	54.3
1138	2158	942	960	52.1	52.6	4658	1497	1480	50.3	50	1.9	556	77	44.6	38	54.1

1139	2159	13040	13059	50.9	50	4659	13312	13294	51	52.6	0.1	273	75.6	44.3	38	53.3
1140	2160	18696	18715	51.7	50	4660	19476	19453	53.5	41.7	1.8	781	75.6	40.3	38	53.5
1141	2161	18696	18715	51.7	50	4661	19476	19456	50.5	42.9	1.3	781	75.6	40.3	38	53.1
1142	2162	3232	3251	50.3	50	4662	3503	3484	51.5	50	1.1	272	74.6	41.9	38	52.4
1143	2163	3031	3051	51.3	52.4	4663	3646	3625	52	40.9	0.7	616	76.4	42.9	38	54
1144	2164	9130	9150	51.3	42.9	4664	9560	9541	50.9	45	0.4	431	75.3	41.3	38	53
1145	2165	18224	18243	53.1	50	4665	18696	18672	53.9	40	0.8	473	75.7	42.1	38	54
1146	2166	18224	18243	53.1	50	4666	18696	18673	53.4	41.7	0.3	473	75.7	42.1	38	54
1147	2167	18225	18243	51.4	52.6	4667	18697	18679	51.9	52.6	0.5	473	75.8	42.3	38	53.6
1148	2168	9130	9150	51.3	42.9	4668	9560	9540	51.6	42.9	0.3	431	75.3	41.3	38	53.2
1149	2169	8866	8885	51.1	45	4669	9252	9235	50.1	50	1	387	75.1	41.3	38	52.7
1150	2170	9130	9150	51.3	42.9	4670	9559	9539	50.6	42.9	0.7	430	75.3	41.4	38	53
1151	2171	12267	12290	54.5	41.7	4671	12501	12480	53.5	45.5	1	235	74.3	42.1	38	53.2
1152	2172	3427	3446	52.7	50	4672	3650	3631	53.1	50	0.4	224	74.3	42.4	38	52.9
1153	2173	3427	3446	52.7	50	4673	3648	3628	52.3	42.9	0.4	222	74	41.9	38	52.6
1154	2174	26039	26058	54	55	4674	26184	26164	52.4	42.9	1.6	146	71.8	40.4	38	51.1
1155	2175	3230	3249	50.1	45	4675	3503	3484	51.5	50	1.4	274	74.5	41.6	38	52.3
1156	2176	3230	3249	50.1	45	4676	3504	3485	50.4	45	0.3	275	74.4	41.5	38	52.2
1157	2177	3427	3446	52.7	50	4677	3646	3625	52	40.9	0.6	220	74	41.8	38	52.5
1158	2178	3429	3449	50.4	42.9	4678	3647	3628	50.6	45	0.2	219	73.9	41.6	38	51.9
1159	2179	3429	3449	50.4	42.9	4679	3646	3625	52	40.9	1.6	218	73.7	41.3	38	51.8
1160	2180	8866	8885	51.1	45	4680	9249	9231	50.8	47.4	0.3	384	75.1	41.4	38	52.9
1161	2181	3428	3449	52.8	45.5	4681	3650	3631	53.1	50	0.3	223	74.2	42.2	38	52.8
1162	2182	18077	18098	52.9	50	4682	18696	18672	53.9	40	1	620	76.2	42.4	38	54.3
1163	2183	18078	18098	51.5	47.6	4683	18696	18673	53.4	41.7	1.9	619	76.2	42.3	38	53.9
1164	2184	8866	8885	51.1	45	4684	9249	9230	51.5	45	0.4	384	75.1	41.4	38	53
1165	2185	3229	3248	50.6	50	4685	3503	3484	51.5	50	0.8	275	74.6	41.8	38	52.5
1166	2186	12267	12290	54.5	41.7	4686	12495	12476	52.7	45	1.9	229	74.1	41.9	38	52.8
1167	2187	8220	8240	54	47.6	4687	8929	8910	54.5	55	0.4	710	75.4	40	38	54.1
1168	2188	18080	18098	51.2	52.6	4688	18238	18219	50.3	45	0.9	159	74	44.7	38	52
1169	2189	18080	18098	51.2	52.6	4689	18239	18220	50	45	1.2	160	73.9	44.4	38	51.8
1170	2190	18080	18098	51.2	52.6	4690	18697	18679	51.9	52.6	0.7	618	76.3	42.6	38	53.8
1171	2191	18076	18097	53.1	45.5	4691	18712	18693	54.8	55	1.7	637	76.3	42.5	38	54.4
1172	2192	8866	8885	51.1	45	4692	9245	9226	50	45	1.1	380	75	41.1	38	52.6
1173	2193	943	961	50.3	47.4	4693	1498	1481	51	50	0.8	556	76.9	44.4	38	54
1174	2194	18075	18095	50.6	47.6	4694	18642	18622	50.5	42.9	0.1	568	76.2	42.6	38	53.6
1175	2195	18075	18095	50.6	47.6	4695	18662	18641	50.4	40.9	0.2	588	76.3	42.7	38	53.6
1176	2196	8866	8885	51.1	45	4696	9107	9086	51.6	45.5	0.5	242	74.1	41.3	38	52.3
1177	2197	943	961	50.3	47.4	4697	1497	1480	50.3	50	0	555	76.9	44.5	38	54
1178	2198	7400	7417	50.2	50	4698	8190	8172	50.3	47.4	0.1	791	76.4	42.2	38	53.6
1179	2199	13039	13058	51.8	50	4699	13314	13297	51	50	0.9	276	75.7	44.6	38	53.4
1180	2200	7725	7743	50.8	47.4	4700	8187	8167	50.4	42.9	0.5	463	75.6	41.9	38	53.1
1181	2201	18074	18094	51.1	42.9	4701	18642	18622	50.5	42.9	0.5	569	76.2	42.5	38	53.6
1182	2202	25782	25805	52.1	41.7	4702	26174	26153	51	40.9	1.1	393	74.8	40.5	38	52.8
1183	2203	9131	9151	50.4	42.9	4703	9560	9541	50.9	45	0.5	430	75.3	41.4	38	52.9
1184	2204	25782	25805	52.1	41.7	4704	26183	26162	52.8	45.5	0.7	402	74.7	40.3	38	53.1
1185	2205	9131	9151	50.4	42.9	4705	9560	9540	51.6	42.9	1.2	430	75.3	41.4	38	52.9
1186	2206	7725	7743	50.8	47.4	4706	8188	8168	50.4	42.9	0.5	464	75.6	41.8	38	53.1
1187	2207	985	1004	51.1	50	4707	1494	1476	50.7	47.4	0.4	510	76.5	43.7	38	53.9
1188	2208	13039	13058	51.8	50	4708	13323	13304	51.1	45	0.7	285	75.8	44.6	38	53.5
1189	2209	9131	9151	50.4	42.9	4709	9559	9539	50.6	42.9	0.3	429	75.3	41.5	38	52.9

1190	2210	12352	12375	52.9	41.7	4710	12499	12480	51.8	45	1.1	148	73.3	43.9	38	52
1191	2211	3225	3244	52.4	55	4711	3646	3625	52	40.9	0.4	422	75.4	41.7	38	53.5
1192	2212	25676	25697	51.9	40.9	4712	25784	25765	53.3	50	1.4	109	70.3	40.4	38	49.9
1193	2213	25363	25381	51.1	52.6	4713	25548	25531	51.1	50	0	186	73.7	42.5	38	52
1194	2214	25363	25381	51.1	52.6	4714	25645	25626	50.8	45	0.4	283	74.2	40.6	38	52.3
1195	2215	18074	18093	50.3	45	4715	18642	18622	50.5	42.9	0.2	569	76.2	42.5	38	53.5
1196	2216	3225	3244	52.4	55	4716	3647	3628	50.6	45	1.8	423	75.5	41.8	38	53.1
1197	2217	3225	3244	52.4	55	4717	3650	3631	53.1	50	0.7	426	75.5	42	38	53.7
1198	2218	12352	12375	52.9	41.7	4718	12494	12476	52.2	47.4	0.6	143	73.2	44.1	38	52
1199	2219	13039	13058	51.8	50	4719	13326	13306	50.7	42.9	1.2	288	75.8	44.4	38	53.4
1200	2220	7617	7636	50.9	50	4720	8188	8169	50.5	45	0.5	572	76.1	42.3	38	53.5
1201	2221	988	1006	52.2	52.6	4721	1697	1678	50.3	45	2	710	76.9	43.8	38	54
1202	2222	12232	12250	51.9	52.6	4722	12739	12719	50.3	42.9	1.7	508	75.8	42.1	38	53.3
1203	2223	12232	12250	51.9	52.6	4723	12739	12718	51	40.9	1	508	75.8	42.1	38	53.5
1204	2224	988	1006	52.2	52.6	4724	1697	1677	51	42.9	1.2	710	76.9	43.8	38	54.2
1205	2225	8867	8886	50.7	50	4725	9341	9322	51.1	50	0.5	475	75.7	41.9	38	53.3
1206	2226	3223	3242	51.8	55	4726	3650	3631	53.1	50	1.3	428	75.6	42.1	38	53.6
1207	2227	8867	8886	50.7	50	4727	9340	9319	50.8	45.5	0.1	474	75.6	41.8	38	53.2
1208	2228	988	1006	52.2	52.6	4728	1697	1676	51.7	40.9	0.6	710	76.9	43.8	38	54.4
1209	2229	988	1006	52.2	52.6	4729	1694	1673	51.7	40.9	0.5	707	76.9	43.8	38	54.5
1210	2230	988	1006	52.2	52.6	4730	1494	1476	50.7	47.4	1.5	507	76.5	43.8	38	53.9
1211	2231	9931	9950	50.2	45	4731	10455	10434	51.1	40.9	1	525	75.3	40.6	38	52.8
1212	2232	3224	3242	50.5	52.6	4732	3646	3625	52	40.9	1.5	423	75.4	41.6	38	53
1213	2233	3224	3242	50.5	52.6	4733	3647	3628	50.6	45	0.1	424	75.4	41.7	38	53.1
1214	2234	3016	3036	50.2	42.9	4734	3187	3166	50.3	45.5	0.1	172	74.6	45.3	38	52.4
1215	2235	24559	24579	52	52.4	4735	25182	25164	51.4	47.4	0.6	624	76	41.8	38	53.7
1216	2236	1782	1802	53.3	47.6	4736	1881	1861	54.5	52.4	1.2	100	72.4	47	38	51.8
1217	2237	7880	7900	50.3	42.9	4737	8188	8169	50.5	45	0.1	309	74.8	41.7	38	52.6
1218	2238	8861	8880	50.2	45	4738	9248	9229	50.1	45	0	388	75	41	38	52.6
1219	2239	8868	8889	50.4	40.9	4739	9312	9293	50.6	45	0.1	445	75.3	41.3	38	53
1220	2240	17790	17813	54.3	41.7	4740	18220	18201	56.1	55	1.8	431	74.9	40.4	38	53.8
1221	2241	24569	24590	56.6	54.5	4741	25184	25164	55.9	52.4	0.7	616	75.9	41.7	38	55
1222	2242	13176	13196	51.4	47.6	4742	13328	13307	51.2	45.5	0.2	153	73.4	43.8	38	51.9
1223	2243	8861	8880	50.2	45	4743	9254	9236	50.6	47.4	0.4	394	74.9	40.9	38	52.6
1224	2244	24622	24643	57.1	54.5	4744	25400	25377	57.2	50	0.1	779	75.7	40.7	38	55.2
1225	2245	8868	8889	50.4	40.9	4745	9256	9237	50.8	45	0.4	389	75	41.1	38	52.7
1226	2246	3361	3381	50.5	42.9	4746	3497	3478	51.3	50	0.8	137	74.1	46.7	38	52.1
1227	2247	4593	4613	51.5	47.6	4747	4711	4693	50.4	47.4	1.1	119	71.5	42	38	50.2
1228	2248	19911	19930	50.7	50	4748	20615	20597	50.6	47.4	0.1	705	75.5	40.3	38	53.1
1229	2249	3221	3239	51.5	52.6	4749	3497	3478	51.3	50	0.2	277	74.6	41.9	38	52.7
1230	2250	3223	3241	50.2	52.6	4750	3504	3485	50.4	45	0.2	282	74.8	42.2	38	52.5
1231	2251	3223	3241	50.2	52.6	4751	3503	3484	51.5	50	1.2	281	74.9	42.3	38	52.6
1232	2252	3360	3380	51.4	42.9	4752	3504	3485	50.4	45	1	145	74.2	46.2	38	52.2
1233	2253	4593	4613	51.5	47.6	4753	4711	4692	51.2	45	0.3	119	71.5	42	38	50.5
1234	2254	4593	4613	51.5	47.6	4754	4710	4691	50.2	45	1.4	118	71.6	42.4	38	50.2
1235	2255	3016	3036	50.2	42.9	4755	3186	3165	50.4	40.9	0.2	171	74.4	45	38	52.3
1236	2256	29182	29206	55.4	44	4756	29301	29282	55.3	55	0.1	120	73.4	46.7	38	53.1
1237	2257	29183	29206	52.9	41.7	4757	29306	29287	54.6	55	1.7	124	73.3	46	38	52.3
1238	2258	29186	29206	51.3	42.9	4758	29298	29279	52.6	55	1.3	113	72.8	46	38	51.5
1239	2259	16979	17000	52.6	50	4759	17483	17465	54.4	52.6	1.8	505	75.9	42.2	38	54
1240	2260	29182	29205	54.6	41.7	4760	29298	29279	52.6	55	1.9	117	73.1	46.2	38	52

1241	2261	16981	17000	51.3	50	4761	17111	17090	51.1	40.9	0.2	131	74.5	48.1	38	52.6
1242	2262	13177	13197	50.3	42.9	4762	13949	13932	51.6	50	1.3	773	75.8	41	38	53.3
1243	2263	8867	8887	52.3	47.6	4763	9252	9234	51.4	52.6	0.9	386	75.1	41.5	38	53.1
1244	2264	24420	24440	50.8	42.9	4764	25081	25063	52.4	52.6	1.6	662	75.7	40.9	38	53.3
1245	2265	7727	7745	50.8	47.4	4765	8188	8169	50.5	45	0.4	462	75.6	41.8	38	53.1
1246	2266	2387	2405	51.6	52.6	4766	3055	3036	50.6	50	1.1	669	76.7	43.3	38	53.9
1247	2267	2671	2692	52.1	40.9	4767	3055	3036	50.6	50	1.5	385	74.8	40.5	38	52.6
1248	2268	29182	29202	51.2	42.9	4768	29298	29279	52.6	55	1.4	117	73.1	46.2	38	51.6
1249	2269	24418	24439	52.9	45.5	4769	25081	25063	52.4	52.6	0.5	664	75.7	41.1	38	53.8
1250	2270	12373	12391	50.8	47.4	4770	12992	12974	51.2	52.6	0.4	620	76.5	43.1	38	53.9
1251	2271	29179	29199	51.4	42.9	4771	29298	29279	52.6	55	1.2	120	73.4	46.7	38	51.9
1252	2272	1783	1803	54.2	47.6	4772	1882	1861	56	50	1.8	100	72.4	47	38	52
1253	2273	12373	12391	50.8	47.4	4773	12498	12480	50	47.4	0.7	126	72.8	44.4	38	51
1254	2274	7728	7746	51.7	52.6	4774	8190	8172	50.3	47.4	1.4	463	75.6	41.9	38	53.1
1255	2275	16875	16896	52.2	45.5	4775	17064	17045	51.4	50	0.8	190	74.5	44.2	38	52.7
1256	2276	1402	1425	52.8	41.7	4776	2103	2082	52	45.5	0.8	702	76.7	43.3	38	54.4
1257	2277	28971	28993	51.9	43.5	4777	29358	29339	52.8	50	0.9	388	76.3	44.3	38	54.1
1258	2278	24380	24399	55	55	4778	25080	25061	54.1	50	1	701	75.9	41.4	38	54.5
1259	2279	24380	24399	55	55	4779	25080	25062	53.5	52.6	1.6	701	75.9	41.4	38	54.3
1260	2280	3168	3189	51	45.5	4780	3497	3478	51.3	50	0.3	330	75.3	42.4	38	53.1
1261	2281	1402	1426	54.1	40	4781	1626	1602	56.1	44	1.9	225	76.4	47.6	38	54.8
1262	2282	24379	24398	55	55	4782	25080	25061	54.1	50	1	702	75.9	41.3	38	54.4
1263	2283	24379	24398	55	55	4783	25080	25062	53.5	52.6	1.6	702	75.9	41.3	38	54.3
1264	2284	16875	16895	51.6	47.6	4784	17062	17045	50.2	50	1.4	188	74.4	44.1	38	52.2
1265	2285	12726	12746	51.3	47.6	4785	12998	12979	50.1	45	1.2	273	75.2	43.2	38	52.7
1266	2286	24378	24397	55	55	4786	24517	24494	53.2	41.7	1.8	140	72.7	42.9	38	51.9
1267	2287	24378	24397	55	55	4787	25080	25061	54.1	50	1	703	75.9	41.3	38	54.4
1268	2288	28939	28961	55.2	47.8	4788	29306	29285	56.7	54.5	1.5	368	76.6	45.1	38	55.3
1269	2289	28940	28961	53.1	45.5	4789	29306	29287	54.6	55	1.5	367	76.5	45	38	54.6
1270	2290	28941	28961	51.6	42.9	4790	29298	29279	52.6	55	1	358	76.3	44.7	38	54
1271	2291	28178	28200	52	43.5	4791	28284	28265	52.9	50	0.9	107	74.7	51.4	38	53
1272	2292	28941	28961	51.6	42.9	4792	29358	29339	52.8	50	1.2	418	76.5	44.5	38	54.2
1273	2293	24378	24397	55	55	4793	25080	25062	53.5	52.6	1.6	703	75.9	41.3	38	54.2
1274	2294	28938	28960	56.1	47.8	4794	29306	29285	56.7	54.5	0.6	369	76.5	45	38	55.5
1275	2295	12234	12252	50.6	47.4	4795	12498	12480	50	47.4	0.5	265	74.7	42.3	38	52.4
1276	2296	28939	28960	54.7	50	4796	29306	29287	54.6	55	0.1	368	76.6	45.1	38	55.1
1277	2297	28140	28158	54.1	52.6	4797	28411	28393	52.9	52.6	1.1	272	78.8	52.2	38	56.2
1278	2298	28941	28960	50.9	45	4798	29298	29279	52.6	55	1.7	358	76.3	44.7	38	53.8
1279	2299	28140	28158	54.1	52.6	4799	28416	28396	52.4	47.6	1.7	277	78.8	52	38	56
1280	2300	28941	28960	50.9	45	4800	29358	29339	52.8	50	1.9	418	76.5	44.5	38	53.9
1281	2301	24179	24200	53.3	40.9	4801	24815	24791	54.5	40	1.2	637	75.8	41.3	38	54.1
1282	2302	28938	28956	50.8	47.4	4802	29298	29279	52.6	55	1.8	361	76.4	44.9	38	53.8
1283	2303	12726	12746	51.3	47.6	4803	12992	12974	51.2	52.6	0.1	267	75.2	43.4	38	53.1
1284	2304	16874	16893	52.1	50	4804	17062	17045	50.2	50	1.9	189	74.6	44.4	38	52.3
1285	2305	1352	1371	56.1	55	4805	1484	1464	54.3	47.6	1.8	133	74.9	48.9	38	53.8
1286	2306	11540	11561	53.8	45.5	4806	11983	11965	53	52.6	0.7	444	75.1	40.8	38	53.6
1287	2307	24179	24199	52.7	42.9	4807	24815	24792	53.4	41.7	0.7	637	75.8	41.3	38	53.9
1288	2308	16555	16572	50.3	50	4808	16777	16758	51.5	50	1.2	223	73.6	40.8	38	51.7
1289	2309	24178	24198	52.7	42.9	4809	24815	24791	54.5	40	1.8	638	75.7	41.2	38	53.9
1290	2310	3192	3213	51.8	45.5	4810	3650	3631	53.1	50	1.3	459	75.7	42	38	53.6
1291	2311	3192	3213	51.8	45.5	4811	3647	3628	50.6	45	1.2	456	75.6	41.9	38	53.2

1292	2312	24174	24195	52.5	40.9	4812	24815	24792	53.4	41.7	0.9	642	75.8	41.3	38	53.9
1293	2313	16553	16571	53.4	52.6	4813	16780	16760	51.4	42.9	2	228	73.7	40.8	38	52.1
1294	2314	16550	16568	54.1	52.6	4814	17041	17023	53.5	52.6	0.6	492	75.9	42.3	38	54.3
1295	2315	3192	3213	51.8	45.5	4815	3646	3625	52	40.9	0.2	455	75.5	41.8	38	53.5
1296	2316	16551	16568	51.1	50	4816	16777	16758	51.5	50	0.4	227	73.9	41.4	38	52.2
1297	2317	12373	12391	50.8	47.4	4817	12998	12979	50.1	45	0.7	626	76.4	43	38	53.6
1298	2318	28868	28887	50.7	45	4818	29414	29395	50.5	50	0.2	547	77	44.8	38	54.2
1299	2319	24028	24047	53.8	50	4819	24815	24791	54.5	40	0.7	788	76.3	42	38	54.6
1300	2320	2427	2445	52.1	52.6	4820	3056	3038	50.8	52.6	1.3	630	76.4	42.9	38	53.8
1301	2321	28867	28886	53.2	50	4821	29306	29288	53.5	52.6	0.3	440	76.9	45.2	38	54.9
1302	2322	24021	24044	52.8	41.7	4822	24815	24791	54.5	40	1.6	795	76.2	41.9	38	54.3
1303	2323	28867	28885	51.5	52.6	4823	29414	29395	50.5	50	0.9	548	77.1	44.9	38	54.2
1304	2324	12369	12388	50.6	45	4824	13155	13137	52.1	52.6	1.5	787	76.8	43.3	38	54
1305	2325	27368	27392	58.2	48	4825	27467	27443	59.4	48	1.2	100	71.2	44	38	52.4
1306	2326	27369	27392	57.2	50	4826	27468	27444	58.4	44	1.2	100	71.2	44	38	52.1
1307	2327	27369	27392	57.2	50	4827	27468	27445	58.1	45.8	0.8	100	71.2	44	38	52.1
1308	2328	23841	23863	53.7	47.8	4828	24022	24003	55.5	55	1.7	182	74.4	44.5	38	53.3
1309	2329	3192	3213	51.8	45.5	4829	3497	3478	51.3	50	0.5	306	75	42.2	38	53
1310	2330	23843	23863	50.3	42.9	4830	24526	24506	50.3	42.9	0	684	76.1	41.8	38	53.4
1311	2331	27366	27389	56.1	45.8	4831	27465	27443	56.4	47.8	0.3	100	71.2	44	38	51.8
1312	2332	27366	27389	56.1	45.8	4832	27465	27444	55.6	45.5	0.6	100	71.2	44	38	51.6
1313	2333	27366	27389	56.1	45.8	4833	27465	27445	55.1	47.6	1	100	71.2	44	38	51.5
1314	2334	16549	16567	54.9	52.6	4834	16779	16758	53.5	50	1.4	231	74	41.6	38	53
1315	2335	27369	27389	52.5	47.6	4835	27468	27448	53.7	47.6	1.1	100	71.2	44	38	50.7
1316	2336	27369	27389	52.5	47.6	4836	27468	27449	52.6	45	0	100	71.2	44	38	50.7
1317	2337	27369	27389	52.5	47.6	4837	27468	27450	51.9	47.4	0.7	100	71.2	44	38	50.5
1318	2338	28654	28672	50.6	52.6	4838	29412	29393	50.3	45	0.2	759	77.9	46.1	38	54.7
1319	2339	2429	2447	50.2	47.4	4839	3053	3034	50.3	50	0.1	625	76.3	42.6	39	53.6
1320	2340	1442	1461	51.6	55	4840	1697	1676	51.7	40.9	0	256	75.8	45.3	39	53.7
1321	2341	1442	1461	51.6	55	4841	1697	1677	51	42.9	0.6	256	75.8	45.3	39	53.5
1322	2342	1442	1461	51.6	55	4842	1697	1678	50.3	45	1.3	256	75.8	45.3	39	53.3
1323	2343	3214	3233	51.1	50	4843	3504	3485	50.4	45	0.7	291	74.8	41.9	39	52.6
1324	2344	3214	3233	51.1	50	4844	3503	3484	51.5	50	0.4	290	74.8	42.1	39	52.8
1325	2345	27374	27392	50.6	47.4	4845	27674	27653	52.5	40.9	1.9	301	74.1	40.2	39	52.2
1326	2346	9930	9949	52.2	50	4846	10670	10649	51.3	40.9	0.9	741	75.8	40.9	39	53.5
1327	2347	1442	1461	51.6	55	4847	2103	2083	50.6	42.9	1	662	76.7	43.4	39	53.9
1328	2348	8867	8887	52.3	47.6	4848	9375	9354	50.4	40.9	2	509	75.7	41.8	39	53.2
1329	2349	16367	16386	51.4	50	4849	16775	16755	51.1	42.9	0.3	409	75	40.8	39	52.9
1330	2350	18081	18100	51.7	50	4850	18702	18685	50.2	50	1.5	622	76.2	42.4	39	53.5
1331	2351	18083	18102	50.6	45	4851	18702	18685	50.2	50	0.4	620	76.1	42.3	39	53.4
1332	2352	18094	18113	51	50	4852	18702	18685	50.2	50	0.8	609	76.1	42.2	39	53.4
1333	2353	8865	8884	50.4	45	4853	9254	9236	50.6	47.4	0.2	390	75	41	39	52.7
1334	2354	16367	16386	51.4	50	4854	16774	16754	50.4	42.9	1	408	75	40.9	39	52.7
1335	2355	18008	18028	53	52.4	4855	18220	18202	54.8	52.6	1.9	213	74.4	43.2	39	53.1
1336	2356	27369	27389	52.5	47.6	4856	27674	27653	52.5	40.9	0.1	306	74.3	40.5	39	52.9
1337	2357	16367	16386	51.4	50	4857	16774	16753	51.1	40.9	0.3	408	75	40.9	39	52.9
1338	2358	1442	1461	51.6	55	4858	2113	2094	50.1	45	1.5	672	76.7	43.3	39	53.8
1339	2359	7876	7895	51.5	45	4859	8190	8172	50.3	47.4	1.2	315	75.1	42.2	39	52.7
1340	2360	18696	18715	51.7	50	4860	19482	19463	50.1	45	1.7	787	75.6	40.3	39	53
1341	2361	12370	12388	50.1	47.4	4861	12911	12892	50.5	50	0.4	542	76.1	42.4	39	53.4
1342	2362	887	905	50.1	47.4	4862	1493	1473	52	47.6	1.9	607	77.1	44.6	39	54.1

1343	2363	16367	16387	51.8	47.6	4863	16774	16751	53.6	41.7	1.8	408	75	40.9	39	53.2
1344	2364	16378	16397	50.4	45	4864	17111	17090	51.1	40.9	0.7	734	76.3	42.2	39	53.6
1345	2365	16378	16397	50.4	45	4865	16781	16761	51.3	47.6	0.8	404	75.1	41.1	39	52.8
1346	2366	1402	1425	52.8	41.7	4866	1501	1478	54.6	41.7	1.8	100	72	46	39	51.3
1347	2367	16378	16397	50.4	45	4867	16777	16758	51.5	50	1	400	75	41	39	52.7
1348	2368	16378	16397	50.4	45	4868	16775	16756	50.3	45	0.1	398	75	41	39	52.7
1349	2369	16378	16397	50.4	45	4869	16775	16755	51.1	42.9	0.6	398	75	41	39	52.7
1350	2370	16378	16397	50.4	45	4870	16774	16754	50.4	42.9	0	397	75	41.1	39	52.7
1351	2371	16378	16397	50.4	45	4871	16774	16753	51.1	40.9	0.7	397	75	41.1	39	52.8
1352	2372	16378	16397	50.4	45	4872	16774	16752	52.2	43.5	1.8	397	75	41.1	39	52.8
1353	2373	10250	10274	51.6	40	4873	10608	10589	51	50	0.6	359	74.6	40.4	39	52.6
1354	2374	16548	16566	54.9	52.6	4874	17112	17090	53.3	43.5	1.6	565	76.3	42.8	39	54.5
1355	2375	19709	19730	51.3	40.9	4875	19922	19902	50	42.9	1.2	214	73.8	41.6	39	51.8
1356	2376	3218	3237	50.5	45	4876	3504	3485	50.4	45	0.1	287	74.7	41.8	39	52.5
1357	2377	3218	3237	50.5	45	4877	3503	3484	51.5	50	0.9	286	74.7	42	39	52.6
1358	2378	19709	19730	51.3	40.9	4878	19920	19899	50.2	40.9	1.1	212	73.7	41.5	39	51.8
1359	2379	1402	1422	50.2	42.9	4879	1501	1480	51.9	40.9	1.7	100	72	46	39	50.6
1360	2380	1402	1422	50.2	42.9	4880	1501	1481	51.2	42.9	1.1	100	72	46	39	50.6
1361	2381	8867	8886	50.7	50	4881	9249	9230	51.5	45	0.9	383	75.2	41.5	39	52.9
1362	2382	19794	19813	50	50	4882	19928	19908	52	52.4	2	135	72.8	43.7	39	51.1
1363	2383	8867	8886	50.7	50	4883	9249	9231	50.8	47.4	0.2	383	75.2	41.5	39	52.9
1364	2384	9927	9945	50.8	52.6	4884	10183	10165	51.7	47.4	0.9	257	75.3	44	39	53.1
1365	2385	27366	27384	52.2	52.6	4885	27566	27546	50.7	47.6	1.5	201	74.7	44.3	39	52.6
1366	2386	9927	9945	50.8	52.6	4886	10183	10166	50.9	50	0.1	257	75.3	44	39	53.1
1367	2387	27366	27384	52.2	52.6	4887	27568	27548	50.2	42.9	1.9	203	74.6	43.8	39	52.4
1368	2388	27366	27384	52.2	52.6	4888	27571	27551	51.4	42.9	0.8	206	74.5	43.7	39	52.7
1369	2389	887	905	50.1	47.4	4889	1483	1465	50.5	47.4	0.4	597	77	44.6	39	54.1
1370	2390	27366	27384	52.2	52.6	4890	27579	27558	51.1	40.9	1.1	214	74.9	44.4	39	52.9
1371	2391	16549	16567	54.9	52.6	4891	16774	16751	53.6	41.7	1.3	226	74	41.6	39	53
1372	2392	19794	19813	50	50	4892	19916	19895	50.2	40.9	0.2	123	72.1	43.1	39	50.6
1373	2393	16551	16568	51.1	50	4893	17062	17045	50.2	50	0.9	512	76	42.4	39	53.3
1374	2394	12726	12746	51.3	47.6	4894	13155	13137	52.1	52.6	0.8	430	76.4	44	39	53.9
1375	2395	545	564	50.7	50	4895	1171	1153	50.4	47.4	0.3	627	78.2	47.2	39	54.9
1376	2396	887	905	50.1	47.4	4896	1483	1464	51.3	45	1.2	597	77	44.6	39	54.1
1377	2397	9927	9945	50.8	52.6	4897	10356	10336	52.4	47.6	1.6	430	75.6	42.1	39	53.3
1378	2398	887	905	50.1	47.4	4898	1481	1463	50.5	47.4	0.4	595	77	44.5	39	54.1
1379	2399	12726	12746	51.3	47.6	4899	12911	12891	51.2	47.6	0.1	186	73.5	41.9	39	51.9
1380	2400	19795	19814	50.4	45	4900	19917	19896	50.9	45.5	0.5	123	72.1	43.1	39	50.7
1381	2401	27361	27380	52.4	55	4901	27566	27546	50.7	47.6	1.7	206	75.1	45.1	39	52.9
1382	2402	27361	27380	52.4	55	4902	27569	27548	50.9	40.9	1.5	209	74.9	44.5	39	52.8
1383	2403	27361	27380	52.4	55	4903	27571	27551	51.4	42.9	1	211	75	44.5	39	53
1384	2404	8867	8886	50.7	50	4904	9256	9237	50.8	45	0.1	390	75.1	41.3	39	52.9
1385	2405	8373	8391	50.7	47.4	4905	9109	9087	50.5	43.5	0.1	737	75.4	40	39	53
1386	2406	19800	19817	50.4	50	4906	19927	19908	52.1	55	1.7	128	72.6	43.8	39	51
1387	2407	19800	19817	50.4	50	4907	19924	19905	50.1	50	0.3	125	72.2	43.2	39	50.7
1388	2408	16553	16571	53.4	52.6	4908	16774	16751	53.6	41.7	0.3	222	73.7	41	39	52.7
1389	2409	2427	2445	52.1	52.6	4909	3053	3034	50.3	50	1.8	627	76.4	42.7	39	53.6
1390	2410	887	905	50.1	47.4	4910	1479	1460	51.6	50	1.5	593	77.1	44.7	39	54.1
1391	2411	13177	13197	50.3	42.9	4911	13321	13301	50.3	42.9	0	145	73.1	43.4	39	51.3
1392	2412	8374	8395	52.4	45.5	4912	9109	9087	50.5	43.5	1.9	736	75.4	40.1	39	53.1
1393	2413	9926	9944	50.5	52.6	4913	10183	10165	51.7	47.4	1.2	258	75.2	43.8	39	52.9

1394	2414	16562	16580	51.9	52.6	4914	17056	17035	51.8	45.5	0.1	495	75.8	42	39	53.7
1395	2415	16562	16581	52.6	50	4915	17056	17035	51.8	45.5	0.8	495	75.8	42	39	53.7
1396	2416	9926	9944	50.5	52.6	4916	10183	10166	50.9	50	0.4	258	75.2	43.8	39	52.9
1397	2417	13177	13197	50.3	42.9	4917	13325	13305	50.5	47.6	0.2	149	73.3	43.6	39	51.5
1398	2418	10141	10160	51	45	4918	10356	10336	52.4	47.6	1.4	216	73.5	40.7	39	51.8
1399	2419	2823	2844	50.4	45.5	4919	3185	3164	51	45.5	0.5	363	75.6	42.7	39	53.1
1400	2420	19800	19818	52.1	52.6	4920	19916	19895	50.2	40.9	1.9	117	71.7	42.7	39	50.3
1401	2421	8063	8084	51.4	45.5	4921	8189	8170	50.6	50	0.8	127	72.3	43.3	39	50.9
1402	2422	985	1008	56.1	50	4922	1485	1465	56	52.4	0	501	76.5	43.7	39	55.4
1403	2423	985	1008	56.1	50	4923	1485	1466	55.6	55	0.5	501	76.5	43.7	39	55.3
1404	2424	985	1008	56.1	50	4924	1495	1474	55.1	45.5	1	511	76.5	43.6	39	55.2
1405	2425	18017	18036	54.8	55	4925	18231	18209	53.5	47.8	1.3	215	74.5	43.3	39	53.3
1406	2426	985	1008	56.1	50	4926	1497	1476	56.4	50	0.3	513	76.6	43.9	39	55.5
1407	2427	13039	13057	51.1	52.6	4927	13155	13137	52.1	52.6	1	117	73.4	47	39	51.8
1408	2428	985	1008	56.1	50	4928	1498	1478	54.9	47.6	1.2	514	76.5	43.8	39	55.1
1409	2429	988	1006	52.2	52.6	4929	1496	1478	50.4	47.4	1.9	509	76.5	43.8	39	53.8
1410	2430	988	1006	52.2	52.6	4930	1497	1480	50.3	50	2	510	76.6	43.9	39	53.8
1411	2431	19856	19875	50.2	45	4931	20033	20016	50.4	50	0.2	178	74.1	43.8	39	52
1412	2432	988	1006	52.2	52.6	4932	1498	1481	51	50	1.2	511	76.6	43.8	39	54
1413	2433	3361	3382	51.9	45.5	4933	3650	3631	53.1	50	1.2	290	75.7	44.1	39	53.6
1414	2434	8867	8888	52.7	45.5	4934	9365	9347	53	52.6	0.3	499	75.8	42.1	39	54
1415	2435	24921	24938	50.4	50	4935	25645	25626	50.8	45	0.4	725	75.5	40.4	39	53.1
1416	2436	3361	3382	51.9	45.5	4936	3647	3628	50.6	45	1.3	287	75.6	43.9	39	53.2
1417	2437	24635	24653	50.5	52.6	4937	25398	25378	51.1	42.9	0.6	764	75.5	40.3	39	53.1
1418	2438	8867	8888	52.7	45.5	4938	9256	9237	50.8	45	1.9	390	75.1	41.3	39	52.9
1419	2439	18017	18036	54.8	55	4939	18712	18693	54.8	55	0	696	76.4	42.5	39	55
1420	2440	24633	24651	50.1	52.6	4940	25398	25378	51.1	42.9	0.9	766	75.6	40.3	39	53
1421	2441	18011	18032	55.7	54.5	4941	18220	18202	54.8	52.6	0.9	210	74.5	43.3	39	53.7
1422	2442	18014	18032	51	52.6	4942	18223	18206	51.8	50	0.8	210	74.3	42.9	39	52.4
1423	2443	24630	24648	50.8	52.6	4943	25398	25378	51.1	42.9	0.2	769	75.6	40.4	39	53.3
1424	2444	18014	18032	51	52.6	4944	18231	18210	52.2	45.5	1.2	218	74.5	43.1	39	52.5
1425	2445	18014	18032	51	52.6	4945	18233	18214	52	50	1.1	220	74.7	43.6	39	52.7
1426	2446	18014	18032	51	52.6	4946	18233	18215	51.3	52.6	0.4	220	74.7	43.6	39	52.7
1427	2447	18011	18031	54.5	52.4	4947	18220	18201	56.1	55	1.6	210	74.5	43.3	39	53.6
1428	2448	3361	3382	51.9	45.5	4948	3646	3625	52	40.9	0.1	286	75.5	43.7	39	53.5
1429	2449	4658	4677	50.5	50	4949	5306	5288	52.4	52.6	2	649	75.5	40.7	39	53.1
1430	2450	18012	18031	53.2	55	4950	18223	18205	53.3	52.6	0.2	212	74.5	43.4	39	53.2
1431	2451	18012	18031	53.2	55	4951	18712	18693	54.8	55	1.7	701	76.4	42.7	39	54.6
1432	2452	13040	13059	50.9	50	4952	13325	13305	50.5	47.6	0.4	286	75.7	44.4	39	53.3
1433	2453	8867	8888	52.7	45.5	4953	9249	9231	50.8	47.4	1.9	383	75.2	41.5	39	53
1434	2454	24179	24198	51	45	4954	24740	24717	52.5	41.7	1.4	562	76	42.2	39	53.6
1435	2455	18013	18031	50.6	52.6	4955	18229	18209	50.1	42.9	0.5	217	74.4	42.9	39	52.2
1436	2456	8865	8884	50.4	45	4956	9340	9319	50.8	45.5	0.3	476	75.5	41.6	39	53.1
1437	2457	24558	24577	50.7	50	4957	24936	24919	51.8	50	1.1	379	75.8	43	39	53.3
1438	2458	8867	8888	52.7	45.5	4958	9249	9230	51.5	45	1.2	383	75.2	41.5	39	53.2
1439	2459	26039	26058	54	55	4959	26753	26733	54	52.4	0.1	715	76	41.5	39	54.5
1440	2460	26039	26058	54	55	4960	26753	26734	52.6	55	1.4	715	76	41.5	39	54.1
1441	2461	18009	18028	51.6	55	4961	18223	18206	51.8	50	0.1	215	74.5	43.3	39	52.7
1442	2462	24482	24503	51.6	40.9	4962	25080	25062	53.5	52.6	1.8	599	75.5	40.7	39	53.4
1443	2463	8861	8880	50.2	45	4963	9109	9087	50.5	43.5	0.4	249	73.8	40.6	39	51.8
1444	2464	24483	24503	51	42.9	4964	25086	25069	50.3	50	0.6	604	75.5	40.7	39	53

1445	2465	18011	18030	52.9	55	4965	18220	18202	54.8	52.6	2	210	74.5	43.3	39	53.1
1446	2466	24481	24502	51.5	45.5	4966	24815	24792	53.4	41.7	1.9	335	75.5	43	39	53.4
1447	2467	24481	24502	51.5	45.5	4967	25081	25063	52.4	52.6	0.9	601	75.5	40.8	39	53.4
1448	2468	24482	24502	50.3	42.9	4968	25082	25064	51.1	52.6	0.8	601	75.5	40.8	39	53
1449	2469	24482	24502	50.3	42.9	4969	25085	25068	50.3	50	0	604	75.4	40.6	39	53
1450	2470	24482	24502	50.3	42.9	4970	25086	25069	50.3	50	0	605	75.5	40.7	39	53
1451	2471	18011	18030	52.9	55	4971	18223	18206	51.8	50	1.1	213	74.4	43.2	39	52.7
1452	2472	18011	18030	52.9	55	4972	18231	18210	52.2	45.5	0.7	221	74.7	43.4	39	53
1453	2473	18011	18030	52.9	55	4973	18233	18214	52	50	0.9	223	74.9	43.9	39	53.1
1454	2474	18011	18030	52.9	55	4974	18233	18215	51.3	52.6	1.6	223	74.9	43.9	39	52.9
1455	2475	24419	24440	52.3	45.5	4975	24815	24792	53.4	41.7	1.2	397	75.9	43.1	39	53.9
1456	2476	18008	18029	54.5	50	4976	18220	18201	56.1	55	1.6	213	74.4	43.2	39	53.6
1457	2477	24420	24440	50.8	42.9	4977	24527	24507	51	42.9	0.2	108	70.7	41.7	39	49.9
1458	2478	12232	12250	51.9	52.6	4978	12994	12976	50.3	47.4	1.6	763	76.4	42.5	39	53.7
1459	2479	4644	4665	52.5	45.5	4979	5306	5288	52.4	52.6	0.1	663	75.6	40.9	39	53.8
1460	2480	18009	18029	53.3	52.4	4980	18712	18693	54.8	55	1.6	704	76.4	42.6	39	54.6
1461	2481	18010	18029	51.8	50	4981	18223	18205	53.3	52.6	1.5	214	74.4	43	39	52.7
1462	2482	24418	24439	52.9	45.5	4982	24527	24507	51	42.9	1.9	110	71.3	42.7	39	50.3
1463	2483	24418	24439	52.9	45.5	4983	24815	24792	53.4	41.7	0.5	398	75.9	43.2	39	54.1
1464	2484	9351	9370	51.2	50	4984	10017	9999	52.8	52.6	1.6	667	75.7	40.9	39	53.4
1465	2485	18011	18029	51.3	52.6	4985	18229	18209	50.1	42.9	1.2	219	74.4	42.9	39	52.2
1466	2486	13176	13196	51.4	47.6	4986	13314	13297	51	50	0.4	139	73	43.9	39	51.5
1467	2487	3229	3248	50.6	50	4987	3497	3478	51.3	50	0.6	269	74.5	41.6	39	52.4
1468	2488	25772	25793	52.4	40.9	4988	26182	26161	51.2	40.9	1.2	411	74.7	40.1	39	52.8
1469	2489	3229	3248	50.6	50	4989	3500	3481	51.2	50	0.5	272	74.5	41.5	39	52.4
1470	2490	13176	13196	51.4	47.6	4990	13323	13304	51.1	45	0.3	148	73.3	43.9	39	51.8
1471	2491	25771	25790	51.1	45	4991	26183	26163	51.7	42.9	0.6	413	74.8	40.4	39	52.8
1472	2492	24418	24436	50	47.4	4992	24526	24506	50.3	42.9	0.3	109	71.4	43.1	39	50.1
1473	2493	25769	25786	50.3	50	4993	26182	26161	51.2	40.9	0.9	414	74.8	40.3	39	52.6
1474	2494	18009	18028	51.6	55	4994	18231	18210	52.2	45.5	0.6	223	74.7	43.5	39	52.9
1475	2495	18009	18028	51.6	55	4995	18233	18214	52	50	0.4	225	74.9	44	39	53
1476	2496	24418	24436	50	47.4	4996	25082	25064	51.1	52.6	1.1	665	75.8	41.2	39	53.2
1477	2497	18009	18028	51.6	55	4997	18233	18215	51.3	52.6	0.3	225	74.9	44	39	53
1478	2498	24418	24436	50	47.4	4998	25209	25190	50.6	50	0.6	792	76.2	41.9	39	53.5
1479	2499	25363	25381	51.1	52.6	4999	25650	25631	51.3	45	0.1	288	74.2	40.6	39	52.4
1480	2500	25363	25381	51.1	52.6	5000	25651	25634	50.4	50	0.7	289	74.3	40.8	39	52.2
1481	2501	25354	25372	50.9	52.6	5001	25548	25531	51.1	50	0.2	195	74.1	43.1	39	52.2
1482	2502	18005	18024	51.1	50	5002	18223	18206	51.8	50	0.6	219	74.4	42.9	39	52.5
1483	2503	18005	18024	51.1	50	5003	18231	18210	52.2	45.5	1.1	227	74.6	43.2	39	52.7
1484	2504	25354	25372	50.9	52.6	5004	25651	25632	52.7	50	1.8	298	74.6	41.3	39	52.6
1485	2505	18005	18024	51.1	50	5005	18233	18215	51.3	52.6	0.2	229	74.9	43.7	39	52.8
1486	2506	18003	18023	53.5	52.4	5006	18712	18693	54.8	55	1.3	710	76.4	42.7	39	54.7
1487	2507	13176	13196	51.4	47.6	5007	13326	13306	50.7	42.9	0.7	151	73.4	43.7	39	51.7
1488	2508	8868	8889	50.4	40.9	5008	9311	9292	50.7	50	0.3	444	75.4	41.4	39	53
1489	2509	25354	25372	50.9	52.6	5009	25832	25811	52.1	50	1.2	479	75	40.3	39	52.9
1490	2510	8375	8396	51.8	45.5	5010	9109	9087	50.5	43.5	1.2	735	75.4	40	39	53
1491	2511	9918	9938	51.4	47.6	5011	10017	9999	52.8	52.6	1.3	100	72.4	47	39	51.2
1492	2512	8375	8396	51.8	45.5	5012	8933	8916	52.2	50	0.4	559	75.1	40.1	39	53.2
1493	2513	17840	17859	50.8	45	5013	18632	18611	50.2	40.9	0.6	793	76.1	41.5	39	53.4
1494	2514	13040	13059	50.9	50	5014	13155	13138	50.4	50	0.5	116	73.2	46.6	39	51.4
1495	2515	25348	25366	51.2	47.4	5015	25650	25631	51.3	45	0.1	303	74.6	41.3	39	52.7

1496	2516	25348	25366	51.2	47.4	5016	25651	25634	50.4	50	0.7	304	74.7	41.4	39	52.5
1497	2517	13040	13059	50.9	50	5017	13178	13157	50.4	40.9	0.5	139	73.6	45.3	39	51.7
1498	2518	17792	17813	51.6	40.9	5018	18223	18205	53.3	52.6	1.7	432	74.9	40.3	39	53
1499	2519	25348	25366	51.2	47.4	5019	25832	25811	52.1	50	0.9	485	75.1	40.4	39	53
1500	2520	25348	25366	51.2	47.4	5020	25833	25812	51.4	45.5	0.2	486	75	40.3	39	53
1501	2521	25347	25365	52	52.6	5021	25651	25632	52.7	50	0.7	305	74.8	41.6	39	53
1502	2522	8868	8889	50.4	40.9	5022	9252	9234	51.4	52.6	1	385	75.1	41.3	39	52.8
1503	2523	17793	17813	50	42.9	5023	18229	18209	50.1	42.9	0.1	437	74.9	40.3	39	52.5
1504	2524	17793	17813	50	42.9	5024	18231	18211	50.6	47.6	0.6	439	75	40.5	39	52.6
1505	2525	17793	17813	50	42.9	5025	18234	18216	51	52.6	1	442	75.1	40.7	39	52.7
1506	2526	17793	17813	50	42.9	5026	18238	18219	50.3	45	0.2	446	75.1	40.8	39	52.7
1507	2527	17793	17813	50	42.9	5027	18239	18220	50	45	0	447	75.1	40.7	39	52.7
1508	2528	24180	24199	50.3	40	5028	24938	24921	50.4	50	0.1	759	75.8	40.8	39	53.2
1509	2529	25348	25365	50.4	50	5029	25832	25811	52.1	50	1.7	485	75.1	40.4	39	52.8
1510	2530	25068	25085	50.3	50	5030	25182	25164	51.4	47.4	1.1	115	73.3	47	39	51.5
1511	2531	29260	29278	51.3	47.4	5031	29414	29395	50.5	50	0.8	155	74.3	45.8	39	52.3
1512	2532	24179	24198	51	45	5032	24933	24913	51.1	42.9	0.1	755	75.8	40.9	39	53.5
1513	2533	17790	17811	51.6	40.9	5033	18223	18205	53.3	52.6	1.7	434	74.9	40.3	39	53
1514	2534	8063	8084	51.4	45.5	5034	8190	8172	50.3	47.4	1.1	128	72.2	43	39	50.8
1515	2535	24178	24197	50.3	40	5035	24936	24919	51.8	50	1.5	759	75.8	41	39	53.2
1516	2536	17791	17811	50	42.9	5036	18229	18209	50.1	42.9	0.1	439	74.9	40.3	39	52.5
1517	2537	17791	17811	50	42.9	5037	18231	18211	50.6	47.6	0.6	441	75	40.6	39	52.6
1518	2538	24174	24194	50.9	42.9	5038	24740	24717	52.5	41.7	1.5	567	76	42.2	39	53.6
1519	2539	24174	24194	50.9	42.9	5039	24933	24913	51.1	42.9	0.2	760	75.8	40.9	39	53.4
1520	2540	17791	17811	50	42.9	5040	18234	18216	51	52.6	1	444	75.1	40.8	39	52.7
1521	2541	17791	17811	50	42.9	5041	18238	18219	50.3	45	0.2	448	75.1	40.8	39	52.7
1522	2542	17791	17811	50	42.9	5042	18239	18220	50	45	0	449	75.1	40.8	39	52.7
1523	2543	24035	24053	52.2	52.6	5043	24526	24506	50.3	42.9	1.9	492	75.4	41.3	39	53
1524	2544	24035	24053	52.2	52.6	5044	24527	24507	51	42.9	1.2	493	75.4	41.2	39	53.2
1525	2545	17607	17628	52.3	40.9	5045	18231	18209	53.5	47.8	1.2	625	75.2	40	39	53.4
1526	2546	29196	29216	52.5	47.6	5046	29358	29339	52.8	50	0.3	163	74.9	46.6	39	53.3
1527	2547	17608	17628	50.9	42.9	5047	18231	18211	50.6	47.6	0.4	624	75.2	40.1	39	52.9
1528	2548	8868	8889	50.4	40.9	5048	9248	9229	50.1	45	0.3	381	75	41.2	39	52.7
1529	2549	17608	17628	50.9	42.9	5049	18234	18216	51	52.6	0	627	75.3	40.2	39	53.1
1530	2550	29196	29215	51.8	50	5050	29358	29339	52.8	50	1	163	74.9	46.6	39	53.1
1531	2551	24023	24044	51.4	40.9	5051	24527	24508	50.5	45	0.9	505	75.4	41	39	53
1532	2552	9409	9428	51.6	45	5052	9989	9968	51	40.9	0.6	581	75.3	40.4	39	53.1
1533	2553	29196	29214	51.1	52.6	5053	29358	29339	52.8	50	1.7	163	74.9	46.6	39	52.8
1534	2554	8861	8880	50.2	45	5054	9257	9238	50.5	45	0.3	397	74.9	40.8	39	52.6
1535	2555	17607	17627	51.6	42.9	5055	18231	18209	53.5	47.8	1.9	625	75.2	40	39	53.2
1536	2556	29195	29213	51.9	52.6	5056	29358	29339	52.8	50	0.9	164	74.8	46.3	39	53
1537	2557	17608	17627	50.2	45	5057	18231	18211	50.6	47.6	0.4	624	75.2	40.1	39	52.8
1538	2558	985	1004	51.1	50	5058	1622	1602	51.6	47.6	0.5	638	77.2	44.7	39	54.4
1539	2559	17608	17627	50.2	45	5059	18234	18216	51	52.6	0.8	627	75.3	40.2	39	52.9
1540	2560	23841	23860	52.1	55	5060	24496	24478	50.7	52.6	1.4	656	76.1	42.1	39	53.6
1541	2561	23841	23860	52.1	55	5061	24498	24479	51.2	50	0.9	658	76.1	42.1	39	53.8
1542	2562	3404	3422	50.5	47.4	5062	3647	3628	50.6	45	0.1	244	74.6	42.6	39	52.5
1543	2563	9349	9367	51.7	52.6	5063	10017	9999	52.8	52.6	1.1	669	75.7	41	39	53.6
1544	2564	23841	23859	50.5	52.6	5064	24496	24478	50.7	52.6	0.2	656	76.1	42.1	39	53.5
1545	2565	25068	25085	50.3	50	5065	25548	25531	51.1	50	0.8	481	75.8	42.2	39	53.3
1546	2566	29186	29205	50.1	40	5066	29414	29395	50.5	50	0.4	229	75.4	45	39	52.9

1547	2567	29182	29204	53.2	43.5	5067	29358	29339	52.8	50	0.4	177	74.6	45.2	39	53.2
1548	2568	23841	23859	50.5	52.6	5068	24498	24479	51.2	50	0.7	658	76.1	42.1	39	53.5
1549	2569	3404	3422	50.5	47.4	5069	3646	3625	52	40.9	1.5	243	74.5	42.4	39	52.4
1550	2570	29183	29204	50.4	40.9	5070	29414	29395	50.5	50	0.2	232	75.4	44.8	39	53
1551	2571	23841	23859	50.5	52.6	5071	24527	24508	50.5	45	0	687	76.1	41.9	39	53.5
1552	2572	23838	23857	50.4	50	5072	24093	24075	50.9	52.6	0.5	256	75.8	45.3	39	53.3
1553	2573	23838	23857	50.4	50	5073	24496	24478	50.7	52.6	0.3	659	76.1	41.9	39	53.4
1554	2574	23838	23857	50.4	50	5074	24498	24479	51.2	50	0.8	661	76.1	41.9	39	53.5
1555	2575	29181	29201	52.4	47.6	5075	29358	29339	52.8	50	0.4	178	74.8	45.5	39	53.2
1556	2576	29181	29201	52.4	47.6	5076	29414	29395	50.5	50	1.9	234	75.6	45.3	39	53.2
1557	2577	29180	29200	51.7	42.9	5077	29358	29339	52.8	50	1.2	179	74.7	45.3	39	52.9
1558	2578	985	1004	51.1	50	5078	1498	1481	51	50	0.1	514	76.5	43.8	39	54
1559	2579	985	1004	51.1	50	5079	1497	1480	50.3	50	0.8	513	76.6	43.9	39	53.8
1560	2580	8859	8879	50	42.9	5080	9254	9236	50.6	47.4	0.6	396	75	40.9	39	52.6
1561	2581	29178	29198	51.4	42.9	5081	29414	29395	50.5	50	0.9	237	75.6	45.1	39	53.2
1562	2582	8859	8879	50	42.9	5082	9340	9319	50.8	45.5	0.7	482	75.5	41.5	39	53
1563	2583	16909	16928	50.8	45	5083	17109	17089	50.4	42.9	0.4	201	74.9	44.8	39	52.7
1564	2584	8794	8813	51.6	45	5084	8919	8901	50.4	47.4	1.2	126	71.8	42.1	39	50.5
1565	2585	8794	8813	51.6	45	5085	8920	8902	52.8	52.6	1.2	127	72	42.5	39	51
1566	2586	985	1004	51.1	50	5086	1496	1478	50.4	47.4	0.7	512	76.5	43.8	39	53.8
1567	2587	18017	18036	54.8	55	5087	18223	18205	53.3	52.6	1.5	207	74.3	43	39	53.1
1568	2588	4593	4613	51.5	47.6	5088	4994	4974	51.2	47.6	0.3	402	76.1	43.5	39	53.7
1569	2589	18017	18036	54.8	55	5089	18220	18201	56.1	55	1.3	204	74.3	43.1	39	53.5
1570	2590	6155	6174	52.1	50	5090	6486	6467	50.8	45	1.2	332	74.5	40.7	39	52.5
1571	2591	6158	6178	51.3	42.9	5091	6486	6467	50.8	45	0.4	329	74.3	40.1	39	52.4
1572	2592	3232	3251	50.3	50	5092	3500	3481	51.2	50	0.8	269	74.5	41.6	39	52.3
1573	2593	3232	3251	50.3	50	5093	3497	3478	51.3	50	1	266	74.5	41.7	39	52.3
1574	2594	28523	28544	51.6	40.9	5094	29298	29279	52.6	55	1	776	78.4	47.3	39	55.5
1575	2595	28965	28984	52.9	55	5095	29358	29339	52.8	50	0.1	394	76.6	44.9	39	54.6
1576	2596	8866	8885	51.1	45	5096	9256	9237	50.8	45	0.3	391	75.1	41.2	39	52.9
1577	2597	28518	28538	51.2	42.9	5097	28672	28654	50.6	52.6	0.7	155	76.7	51.6	39	54
1578	2598	6165	6183	51.2	52.6	5098	6486	6467	50.8	45	0.3	322	74.5	40.7	39	52.5
1579	2599	6264	6283	50.4	50	5099	6483	6463	50.2	42.9	0.2	220	73.8	41.4	39	51.8
1580	2600	18074	18093	50.3	45	5100	18233	18215	51.3	52.6	1	160	73.9	44.4	39	51.9
1581	2601	6271	6291	51.1	47.6	5101	6483	6463	50.2	42.9	0.9	213	73.5	40.8	39	51.6
1582	2602	18074	18093	50.3	45	5102	18231	18210	52.2	45.5	1.9	158	73.5	43.7	39	51.7
1583	2603	6274	6293	50.1	45	5103	6483	6463	50.2	42.9	0.1	210	73.5	41	39	51.6
1584	2604	18074	18093	50.3	45	5104	18223	18206	51.8	50	1.5	150	73.2	43.3	39	51.4
1585	2605	5	23	51.3	52.6	5105	314	296	50.6	47.4	0.6	310	76.8	46.5	39	54
1586	2606	6343	6364	50.7	45.5	5106	6486	6467	50.8	45	0.1	144	71.7	40.3	39	50.5
1587	2607	3800	3820	50.6	42.9	5107	4445	4425	50.6	42.9	0	646	75.4	40.2	39	53
1588	2608	7615	7635	51.1	47.6	5108	7821	7798	52.8	41.7	1.6	207	73.7	41.5	39	52
1589	2609	7723	7741	52.2	52.6	5109	8049	8032	50.4	50	1.8	327	74.9	41.6	39	52.6
1590	2610	1	19	50.1	52.6	5110	314	296	50.6	47.4	0.6	314	76.8	46.5	39	53.9
1591	2611	7725	7742	50	50	5111	7856	7836	51.1	42.9	1.1	132	71.3	40.2	39	50
1592	2612	18074	18094	51.1	42.9	5112	18233	18215	51.3	52.6	0.3	160	73.9	44.4	39	52.1
1593	2613	3168	3189	51	45.5	5113	3503	3484	51.5	50	0.5	336	75.3	42.6	39	53.1
1594	2614	18074	18094	51.1	42.9	5114	18231	18210	52.2	45.5	1.1	158	73.5	43.7	39	51.9
1595	2615	13177	13197	50.3	42.9	5115	13312	13294	51	52.6	0.7	136	72.7	43.4	39	51.1
1596	2616	28190	28209	54.2	55	5116	28671	28652	52.8	55	1.5	482	79.9	52.1	39	56.8
1597	2617	28190	28209	54.2	55	5117	28673	28654	53.5	55	0.7	484	79.9	52.3	39	57.1

1598	2618	28190	28208	51.7	52.6	5118	28671	28652	52.8	55	1	482	79.9	52.1	39	56.5
1599	2619	28190	28208	51.7	52.6	5119	28671	28653	50.2	52.6	1.5	482	79.9	52.1	39	56.1
1600	2620	18074	18094	51.1	42.9	5120	18223	18206	51.8	50	0.7	150	73.2	43.3	39	51.6
1601	2621	28185	28205	53.5	47.6	5121	28284	28265	52.9	50	0.6	100	74.5	52	39	53.1
1602	2622	28187	28205	53.1	52.6	5122	28672	28653	51.8	55	1.2	486	79.9	52.3	39	56.6
1603	2623	2371	2389	50.3	47.4	5123	2900	2881	50.1	45	0.2	530	76.8	44.3	39	53.9
1604	2624	2371	2389	50.3	47.4	5124	3052	3033	50.3	50	0.1	682	76.7	43.4	39	53.9
1605	2625	2371	2389	50.3	47.4	5125	3056	3038	50.8	52.6	0.5	686	76.7	43.4	39	53.9
1606	2626	18074	18095	52.2	45.5	5126	18223	18205	53.3	52.6	1.1	150	73.2	43.3	39	52
1607	2627	2220	2239	51.3	45	5127	2891	2873	50.8	47.4	0.5	672	76.8	43.8	39	54.1
1608	2628	18077	18097	51.5	47.6	5128	18662	18641	50.4	40.9	1.1	586	76.2	42.7	39	53.6
1609	2629	28117	28135	50.6	52.6	5129	28671	28653	50.2	52.6	0.4	555	80	51.9	39	56.1
1610	2630	28116	28134	50.8	47.4	5130	28671	28653	50.2	52.6	0.6	556	79.9	51.8	39	56.1
1611	2631	12232	12250	51.9	52.6	5131	13000	12981	51.1	45	0.8	769	76.5	42.5	39	54
1612	2632	18080	18098	51.2	52.6	5132	18702	18685	50.2	50	1	623	76.2	42.4	39	53.5
1613	2633	12232	12250	51.9	52.6	5133	12999	12980	50.6	40	1.4	768	76.4	42.4	39	53.8
1614	2634	28820	28840	54.8	47.6	5134	29306	29285	56.7	54.5	1.9	487	77.1	45.4	39	55.5
1615	2635	28820	28840	54.8	47.6	5135	29306	29287	54.6	55	0.3	487	77.1	45.4	39	55.5
1616	2636	18080	18098	51.2	52.6	5136	18642	18622	50.5	42.9	0.7	563	76.2	42.6	39	53.6
1617	2637	8865	8884	50.4	45	5137	9249	9231	50.8	47.4	0.4	385	75.1	41.3	39	52.8
1618	2638	8865	8884	50.4	45	5138	9249	9230	51.5	45	1.1	385	75.1	41.3	39	52.8
1619	2639	8865	8884	50.4	45	5139	9109	9087	50.5	43.5	0.1	245	73.9	40.8	39	51.9
1620	2640	28819	28839	56.6	52.4	5140	29306	29285	56.7	54.5	0.2	488	77.2	45.5	39	56.1
1621	2641	9130	9151	52	40.9	5141	9364	9346	53.9	52.6	2	235	74.8	43.4	39	53.1
1622	2642	28820	28839	54.3	50	5142	29306	29287	54.6	55	0.3	487	77.1	45.4	39	55.4
1623	2643	8865	8884	50.4	45	5143	9248	9229	50.1	45	0.3	384	75	41.1	39	52.7
1624	2644	18080	18098	51.2	52.6	5144	18229	18209	50.1	42.9	1.1	150	73.2	43.3	39	51.4
1625	2645	15752	15772	50.8	47.6	5145	16175	16155	51.8	47.6	1	424	75.1	41	39	52.9
1626	2646	12232	12250	51.9	52.6	5146	12498	12480	50	47.4	1.9	267	74.7	42.3	39	52.4
1627	2647	18078	18098	51.5	47.6	5147	18223	18205	53.3	52.6	1.8	146	73	43.2	39	51.6
1628	2648	7833	7853	50.7	47.6	5148	8054	8035	50.4	50	0.2	222	74.6	43.2	39	52.4
1629	2649	230	248	51.2	52.6	5149	713	695	50.7	47.4	0.5	484	79.3	50.6	39	55.8
1630	2650	1472	1491	51.2	45	5150	2153	2134	50.4	45	0.8	682	76.5	42.8	39	53.7
1631	2651	18076	18098	54.4	47.8	5151	18220	18201	56.1	55	1.8	145	73.1	43.4	39	52.6
1632	2652	1442	1461	51.6	55	5152	1694	1673	51.7	40.9	0.1	253	75.9	45.5	39	53.7
1633	2653	28618	28636	52.5	52.6	5153	29358	29339	52.8	50	0.3	741	78.2	47	39	55.6
1634	2654	940	959	56.3	55	5154	1701	1677	54.7	40	1.6	762	77.1	44.2	40	55.5
1635	2655	18076	18097	53.1	45.5	5155	18696	18672	53.9	40	0.8	621	76.2	42.4	40	54.4
1636	2656	940	959	56.3	55	5156	1697	1673	54.4	40	1.9	758	77.2	44.3	40	55.5
1637	2657	3016	3036	50.2	42.9	5157	3188	3167	50.2	40.9	0.1	173	74.5	45.1	40	52.3
1638	2658	18077	18097	51.5	47.6	5158	18696	18673	53.4	41.7	1.9	620	76.2	42.4	40	53.9
1639	2659	18077	18097	51.5	47.6	5159	18697	18679	51.9	52.6	0.3	621	76.2	42.5	40	53.9
1640	2660	9352	9371	50.6	45	5160	9989	9968	51	40.9	0.4	638	75.4	40.3	40	53
1641	2661	6042	6062	50.4	47.6	5161	6374	6353	50	40.9	0.3	333	74.6	40.8	40	52.3
1642	2662	942	960	52.1	52.6	5162	1697	1678	50.3	45	1.8	756	77.2	44.3	40	54.2
1643	2663	942	960	52.1	52.6	5163	1697	1677	51	42.9	1.1	756	77.2	44.3	40	54.4
1644	2664	6042	6062	50.4	47.6	5164	6292	6273	50.8	45	0.4	251	73.9	40.6	40	51.9
1645	2665	942	960	52.1	52.6	5165	1697	1676	51.7	40.9	0.5	756	77.2	44.3	40	54.6
1646	2666	942	960	52.1	52.6	5166	1694	1673	51.7	40.9	0.4	753	77.2	44.4	40	54.7
1647	2667	13176	13196	51.4	47.6	5167	13749	13727	50.5	43.5	0.9	574	76.2	42.7	40	53.6
1648	2668	13176	13196	51.4	47.6	5168	13949	13932	51.6	50	0.2	774	75.9	41.1	40	53.6

1649	2669	942	960	52.1	52.6	5169	1493	1473	52	47.6	0.1	552	76.9	44.4	40	54.5
1650	2670	6042	6062	50.4	47.6	5170	6292	6272	51.5	42.9	1.1	251	73.9	40.6	40	51.9
1651	2671	6042	6062	50.4	47.6	5171	6290	6270	50.9	42.9	0.6	249	73.8	40.6	40	51.9
1652	2672	6042	6062	50.4	47.6	5172	6289	6267	52.2	43.5	1.8	248	73.9	40.7	40	51.9
1653	2673	9402	9420	51.3	47.4	5173	10017	9999	52.8	52.6	1.4	616	75.6	41.1	40	53.5
1654	2674	943	961	50.3	47.4	5174	1697	1678	50.3	45	0	755	77.1	44.2	40	54.2
1655	2675	9139	9159	52.5	47.6	5175	9324	9300	52.9	40	0.4	186	73.9	43	40	52.6
1656	2676	9139	9159	52.5	47.6	5176	9324	9301	52.4	41.7	0.1	186	73.9	43	40	52.6
1657	2677	943	961	50.3	47.4	5177	1697	1677	51	42.9	0.7	755	77.1	44.2	40	54.2
1658	2678	6222	6246	52.2	40	5178	6486	6467	50.8	45	1.4	265	73.8	40	40	52
1659	2679	3895	3914	50.3	45	5179	4608	4590	51.5	52.6	1.2	714	75.5	40.3	40	53
1660	2680	3889	3911	54.2	47.8	5180	4610	4590	53.2	52.4	1.1	722	75.5	40.4	40	53.9
1661	2681	3889	3908	51.3	50	5181	4608	4590	51.5	52.6	0.3	720	75.5	40.4	40	53.4
1662	2682	9139	9159	52.5	47.6	5182	9359	9335	54.5	40	1.9	221	74.7	43.4	40	53.1
1663	2683	943	961	50.3	47.4	5183	1697	1676	51.7	40.9	1.4	755	77.1	44.2	40	54.2
1664	2684	943	961	50.3	47.4	5184	1694	1673	51.7	40.9	1.5	752	77.2	44.3	40	54.2
1665	2685	6302	6321	51.4	50	5185	6483	6463	50.2	42.9	1.2	182	72.9	40.7	40	51.2
1666	2686	9409	9428	51.6	45	5186	10017	9999	52.8	52.6	1.2	609	75.6	41.1	40	53.5
1667	2687	943	961	50.3	47.4	5187	1493	1473	52	47.6	1.7	551	76.8	44.3	40	54
1668	2688	13039	13058	51.8	50	5188	13312	13294	51	52.6	0.8	274	75.7	44.5	40	53.4
1669	2689	3799	3820	52.9	45.5	5189	4565	4542	53.9	41.7	1	767	75.5	40.2	40	53.8
1670	2690	985	1004	51.1	50	5190	1481	1463	50.5	47.4	0.6	497	76.4	43.5	40	53.7
1671	2691	13039	13058	51.8	50	5191	13325	13305	50.5	47.6	1.3	287	75.8	44.6	40	53.3
1672	2692	7615	7635	51.1	47.6	5192	8049	8032	50.4	50	0.8	435	75.7	42.3	40	53.2
1673	2693	7615	7635	51.1	47.6	5193	7853	7833	50.7	47.6	0.4	239	74.4	42.3	40	52.4
1674	2694	3034	3053	50.3	50	5194	3503	3484	51.5	50	1.2	470	76.3	43.4	40	53.6
1675	2695	9140	9159	50.1	45	5195	9334	9315	52.1	50	2	195	74.3	43.6	40	52.1
1676	2696	3799	3819	51.3	47.6	5196	4186	4168	51.8	52.6	0.5	388	75.3	41.8	40	53.2
1677	2697	3799	3819	51.3	47.6	5197	4434	4416	51.5	52.6	0.2	636	75.3	40.3	40	53.2
1678	2698	3799	3819	51.3	47.6	5198	4435	4417	50.5	52.6	0.8	637	75.4	40.3	40	53
1679	2699	7617	7636	50.9	50	5199	8190	8172	50.3	47.4	0.6	574	76.1	42.3	40	53.4
1680	2700	18011	18032	55.7	54.5	5200	18443	18424	55.9	55	0.2	433	76.1	43.2	40	55.1
1681	2701	18013	18032	52.2	55	5201	18696	18672	53.9	40	1.7	684	76.3	42.4	40	54.2
1682	2702	18013	18032	52.2	55	5202	18696	18673	53.4	41.7	1.2	684	76.3	42.4	40	54.2
1683	2703	13177	13197	50.3	42.9	5203	13545	13527	50.3	52.6	0	369	77	46.1	40	54.1
1684	2704	9922	9941	51.3	50	5204	10455	10434	51.1	40.9	0.1	534	75.3	40.6	40	53.1
1685	2705	7617	7636	50.9	50	5205	7853	7833	50.7	47.6	0.3	237	74.4	42.2	40	52.4
1686	2706	13177	13197	50.3	42.9	5206	13329	13308	50.5	40.9	0.2	153	73.2	43.1	40	51.4
1687	2707	18014	18032	51	52.6	5207	18238	18219	50.3	45	0.7	225	74.8	43.6	40	52.5
1688	2708	18014	18032	51	52.6	5208	18239	18220	50	45	0.9	226	74.7	43.4	40	52.4
1689	2709	18014	18032	51	52.6	5209	18697	18679	51.9	52.6	0.9	684	76.3	42.4	40	53.8
1690	2710	7708	7730	50.6	43.5	5210	7853	7833	50.7	47.6	0.1	146	71.8	40.4	40	50.6
1691	2711	9140	9159	50.1	45	5211	9358	9338	51	42.9	0.9	219	74.4	42.9	40	52.2
1692	2712	7723	7741	52.2	52.6	5212	7856	7836	51.1	42.9	1.1	134	71.4	40.3	40	50.4
1693	2713	988	1006	52.2	52.6	5213	1171	1153	50.4	47.4	1.8	184	73.8	42.9	40	51.9
1694	2714	13177	13197	50.3	42.9	5214	13328	13307	51.2	45.5	0.9	152	73.3	43.4	40	51.5
1695	2715	9935	9955	50.4	42.9	5215	10608	10589	51	50	0.6	674	75.8	41.1	40	53.2
1696	2716	985	1008	56.1	50	5216	1484	1463	55.5	50	0.5	500	76.4	43.6	40	55.3
1697	2717	13033	13051	52.1	52.6	5217	13179	13158	50.4	40.9	1.7	147	74.3	46.3	40	52.2
1698	2718	12977	12996	50.2	40	5218	13320	13300	51.4	47.6	1.1	344	76.1	44.2	40	53.4
1699	2719	12977	12996	50.2	40	5219	13321	13301	50.3	42.9	0.1	345	76	44.1	40	53.4

1700	2720	2823	2844	50.4	45.5	5220	3192	3171	51.9	50	1.5	370	75.7	43	40	53.2
1701	2721	18009	18030	54.6	54.5	5221	18443	18424	55.9	55	1.4	435	76.1	43.2	40	54.7
1702	2722	12976	12995	51.1	45	5222	13320	13300	51.4	47.6	0.3	345	76.1	44.3	40	53.7
1703	2723	1046	1064	51.2	47.4	5223	1531	1512	52.7	55	1.5	486	76.7	44.2	40	54.1
1704	2724	12976	12995	51.1	45	5224	13321	13301	50.3	42.9	0.8	346	76.1	44.2	40	53.5
1705	2725	12976	12994	50.3	47.4	5225	13320	13300	51.4	47.6	1.1	345	76.1	44.3	40	53.5
1706	2726	12976	12994	50.3	47.4	5226	13321	13301	50.3	42.9	0	346	76.1	44.2	40	53.5
1707	2727	18011	18030	52.9	55	5227	18696	18672	53.9	40	1	686	76.3	42.4	40	54.4
1708	2728	18011	18030	52.9	55	5228	18696	18673	53.4	41.7	0.5	686	76.3	42.4	40	54.4
1709	2729	18011	18030	52.9	55	5229	18697	18679	51.9	52.6	1	687	76.3	42.5	40	54.1
1710	2730	9140	9159	50.1	45	5230	9374	9353	50.1	40.9	0	235	74.5	42.6	40	52.3
1711	2731	3	23	55.4	52.4	5231	204	185	56.6	55	1.3	202	75	45	40	54.2
1712	2732	15255	15273	50.3	52.6	5232	15761	15741	51.7	47.6	1.4	507	75	40	40	52.7
1713	2733	15255	15273	50.3	52.6	5233	15763	15743	52	47.6	1.7	509	75	40.1	40	52.7
1714	2734	12965	12985	51.2	42.9	5234	13320	13300	51.4	47.6	0.2	356	76.1	44.1	40	53.7
1715	2735	8373	8391	50.7	47.4	5235	9060	9039	50.3	40.9	0.4	688	75.4	40.1	40	53
1716	2736	12962	12980	50.7	47.4	5236	13320	13300	51.4	47.6	0.7	359	76.2	44.3	40	53.6
1717	2737	12938	12957	50.9	45	5237	13155	13137	52.1	52.6	1.2	218	75.4	45.4	40	53.2
1718	2738	2671	2692	52.1	40.9	5238	3190	3169	50.7	45.5	1.5	520	75.6	41.5	40	53.2
1719	2739	2671	2692	52.1	40.9	5239	3192	3171	51.9	50	0.2	522	75.7	41.8	40	53.7
1720	2740	12938	12956	50.1	47.4	5240	13155	13137	52.1	52.6	2	218	75.4	45.4	40	52.9
1721	2741	26421	26441	51.5	42.9	5241	26592	26574	52.4	52.6	0.9	172	72.4	40.1	40	51.2
1722	2742	18006	18028	54.5	52.2	5242	18443	18424	55.9	55	1.4	438	76.1	43.2	40	54.7
1723	2743	26421	26441	51.5	42.9	5243	26656	26635	52.9	45.5	1.4	236	74.2	41.9	40	52.5
1724	2744	3055	3074	51.1	50	5244	3210	3190	50.5	47.6	0.6	156	74.2	45.5	40	52.2
1725	2745	7833	7853	50.7	47.6	5245	8189	8170	50.6	50	0	357	75.6	42.9	40	53.2
1726	2746	26421	26441	51.5	42.9	5246	26658	26640	50.8	47.4	0.7	238	74.1	41.6	40	52.2
1727	2747	9131	9151	50.4	42.9	5247	9328	9310	51	52.6	0.7	198	74.3	43.4	40	52.2
1728	2748	24921	24938	50.4	50	5248	25650	25631	51.3	45	0.9	730	75.5	40.4	40	53.1
1729	2749	24921	24938	50.4	50	5249	25651	25634	50.4	50	0	731	75.6	40.5	40	53.1
1730	2750	9130	9151	52	40.9	5250	9324	9301	52.4	41.7	0.5	195	73.9	42.6	40	52.4
1731	2751	9130	9151	52	40.9	5251	9324	9300	52.9	40	1	195	73.9	42.6	40	52.4
1732	2752	8376	8396	50.6	42.9	5252	9107	9086	51.6	45.5	1	732	75.4	40	40	53.1
1733	2753	11541	11561	50.9	42.9	5253	11727	11708	50.4	45	0.5	187	73	40.6	40	51.3
1734	2754	11540	11561	53.8	45.5	5254	11984	11966	53	52.6	0.7	445	75.1	40.7	40	53.6
1735	2755	2371	2389	50.3	47.4	5255	2672	2654	50.9	52.6	0.5	302	77.1	47.4	40	54.2
1736	2756	2371	2389	50.3	47.4	5256	2998	2977	51.1	40.9	0.8	628	76.7	43.5	40	53.9
1737	2757	11543	11562	50.4	40	5257	11727	11708	50.4	45	0.1	185	72.9	40.5	40	51.2
1738	2758	26040	26061	56.4	54.5	5258	26589	26567	56.1	47.8	0.4	550	75.1	40	40	54.5
1739	2759	11541	11562	51.5	40.9	5259	11984	11966	53	52.6	1.5	444	75	40.5	40	53.1
1740	2760	7728	7746	51.7	52.6	5260	8187	8167	50.4	42.9	1.3	460	75.6	42	40	53.2
1741	2761	26040	26061	56.4	54.5	5261	26657	26634	54.6	41.7	1.9	618	75.5	40.6	40	54.3
1742	2762	2223	2243	50.2	42.9	5262	2675	2656	50.4	50	0.2	453	77	45.3	40	54
1743	2763	2220	2239	51.3	45	5263	2676	2657	50.7	50	0.5	457	76.9	45.1	40	54.1
1744	2764	11541	11560	50.1	45	5264	11727	11707	51.1	42.9	1	187	73	40.6	40	51.2
1745	2765	24559	24580	54.2	54.5	5265	25088	25070	54.5	52.6	0.2	530	75.5	41.1	40	54.2
1746	2766	12233	12251	51.1	52.6	5266	12998	12979	50.1	45	1	766	76.5	42.6	40	53.7
1747	2767	12233	12251	51.1	52.6	5267	12412	12392	50	42.9	1.1	180	73.2	41.7	40	51.4
1748	2768	24562	24580	50.1	52.6	5268	25086	25069	50.3	50	0.2	525	75.4	41	40	52.9
1749	2769	9931	9950	50.2	45	5269	10608	10589	51	50	0.8	678	75.8	41.2	40	53.2
1750	2770	24562	24580	50.1	52.6	5270	25209	25188	52	45.5	1.9	648	76.1	42	40	53.4

1751	2771	24562	24580	50.1	52.6	5271	25209	25189	51.4	47.6	1.3	648	76.1	42	40	53.4
1752	2772	3789	3807	51.8	52.6	5272	4445	4425	50.6	42.9	1.2	657	75.5	40.5	40	53.1
1753	2773	26039	26058	54	55	5273	26656	26634	53.4	43.5	0.6	618	75.5	40.6	40	53.9
1754	2774	26039	26058	54	55	5274	26660	26639	52.5	45.5	1.5	622	75.4	40.5	40	53.7
1755	2775	3789	3807	51.8	52.6	5275	4444	4424	50.6	42.9	1.2	656	75.5	40.5	40	53.1
1756	2776	24559	24579	52	52.4	5276	25086	25069	50.3	50	1.6	528	75.5	41.1	40	53
1757	2777	12235	12253	50.1	52.6	5277	12999	12980	50.6	40	0.5	765	76.4	42.5	40	53.6
1758	2778	24559	24579	52	52.4	5278	25209	25188	52	45.5	0	651	76.1	42.1	40	54
1759	2779	24559	24579	52	52.4	5279	25209	25189	51.4	47.6	0.6	651	76.1	42.1	40	53.8
1760	2780	887	905	50.1	47.4	5280	1499	1482	50.1	50	0.1	613	77.1	44.7	40	54.1
1761	2781	24558	24577	50.7	50	5281	25182	25164	51.4	47.4	0.7	625	76	41.9	40	53.5
1762	2782	26039	26058	54	55	5282	26828	26810	52.9	52.6	1.2	790	76.4	42.4	40	54.5
1763	2783	8866	8885	51.1	45	5283	9597	9577	50.3	42.9	0.8	732	75.8	41.1	40	53.3
1764	2784	7727	7745	50.8	47.4	5284	8049	8032	50.4	50	0.5	323	74.8	41.5	40	52.6
1765	2785	13177	13197	50.3	42.9	5285	13747	13726	50.8	40.9	0.4	571	76.2	42.6	40	53.5
1766	2786	887	905	50.1	47.4	5286	1498	1481	51	50	0.9	612	77.2	44.8	40	54.1
1767	2787	1784	1803	52.5	50	5287	2103	2083	50.6	42.9	2	320	76	44.4	40	53.5
1768	2788	12235	12253	50.1	52.6	5288	12498	12480	50	47.4	0.1	264	74.7	42.4	40	52.4
1769	2789	1784	1802	51.8	52.6	5289	2103	2083	50.6	42.9	1.3	320	76	44.4	40	53.5
1770	2790	887	905	50.1	47.4	5290	1496	1478	50.4	47.4	0.3	610	77.1	44.8	40	54.1
1771	2791	1783	1801	52.9	52.6	5291	2153	2133	52.1	42.9	0.9	371	76	43.7	40	53.9
1772	2792	887	905	50.1	47.4	5292	1494	1476	50.7	47.4	0.6	608	77.1	44.7	40	54.1
1773	2793	3791	3809	51.8	52.6	5293	4445	4425	50.6	42.9	1.2	655	75.5	40.5	40	53.1
1774	2794	17813	17832	50.1	45	5294	18506	18488	51.2	52.6	1.2	694	75.8	41.2	40	53.2
1775	2795	3791	3809	51.8	52.6	5295	4444	4424	50.6	42.9	1.2	654	75.5	40.5	40	53.1
1776	2796	17840	17859	50.8	45	5296	18233	18215	51.3	52.6	0.5	394	74.9	40.9	40	52.8
1777	2797	17840	17859	50.8	45	5297	18233	18214	52	50	1.3	394	74.9	40.9	40	52.8
1778	2798	9930	9949	52.2	50	5298	10356	10336	52.4	47.6	0.2	427	75.6	42.2	40	53.7
1779	2799	15211	15230	50.2	45	5299	15949	15930	51.1	45	0.9	739	75.5	40.3	40	53
1780	2800	98	118	50.6	42.9	5300	253	233	51.8	47.6	1.2	156	74.5	46.2	40	52.4
1781	2801	18004	18023	51.1	50	5301	18233	18214	52	50	0.9	230	75	43.9	40	52.9
1782	2802	24420	24440	50.8	42.9	5302	24936	24919	51.8	50	1	517	75.9	42.2	40	53.5
1783	2803	24420	24440	50.8	42.9	5303	24938	24921	50.4	50	0.4	519	75.8	42	40	53.3
1784	2804	98	118	50.6	42.9	5304	254	235	50	45	0.6	157	74.4	45.9	40	52.2
1785	2805	11540	11557	50.4	50	5305	11727	11707	51.1	42.9	0.7	188	73.1	41	40	51.4
1786	2806	98	118	50.6	42.9	5306	642	622	51.6	47.6	0.9	545	79.1	49.7	40	55.6
1787	2807	15211	15230	50.2	45	5307	15595	15576	50.8	45	0.6	385	75.1	41.3	40	52.7
1788	2808	15255	15273	50.3	52.6	5308	15767	15747	50	42.9	0.3	513	75.1	40.2	40	52.6
1789	2809	12373	12391	50.8	47.4	5309	12911	12891	51.2	47.6	0.4	539	76.1	42.5	40	53.6
1790	2810	18009	18028	51.6	55	5310	18697	18679	51.9	52.6	0.2	689	76.4	42.5	40	54
1791	2811	18009	18028	51.6	55	5311	18696	18673	53.4	41.7	1.8	688	76.3	42.4	40	54
1792	2812	18009	18028	51.6	55	5312	18239	18220	50	45	1.6	231	74.9	43.7	40	52.5
1793	2813	18009	18028	51.6	55	5313	18238	18219	50.3	45	1.4	230	75	43.9	40	52.7
1794	2814	24417	24436	52.6	50	5314	25079	25061	52.7	52.6	0.1	663	75.8	41.3	40	54
1795	2815	10250	10271	50.6	45.5	5315	10356	10336	52.4	47.6	1.8	107	70.8	42.1	40	49.9
1796	2816	1356	1375	53.8	55	5316	1484	1464	54.3	47.6	0.5	129	74.4	48.1	40	53.3
1797	2817	1356	1375	53.8	55	5317	1484	1465	53.8	50	0	129	74.4	48.1	40	53.3
1798	2818	1356	1375	53.8	55	5318	1484	1466	53.1	52.6	0.7	129	74.4	48.1	40	53.1
1799	2819	24418	24436	50	47.4	5319	24560	24542	50.2	47.4	0.1	143	72.4	42	40	50.8
1800	2820	18008	18028	53	52.4	5320	18696	18672	53.9	40	0.9	689	76.3	42.4	40	54.4
1801	2821	25363	25381	51.1	52.6	5321	25646	25627	50.5	45	0.7	284	74.1	40.5	40	52.1

1802	2822	9131	9151	50.4	42.9	5322	9333	9315	52.2	52.6	1.9	203	74.6	43.8	40	52.4
1803	2823	9131	9151	50.4	42.9	5323	9374	9353	50.1	40.9	0.3	244	74.6	42.6	40	52.4
1804	2824	24380	24399	55	55	5324	24582	24560	54.2	52.2	0.9	203	74.4	43.3	40	53.4
1805	2825	19802	19820	53	52.6	5325	19921	19900	51.8	45.5	1.2	120	72.4	44.2	40	51.3
1806	2826	3055	3075	51.8	47.6	5326	3210	3190	50.5	47.6	1.3	156	74.2	45.5	40	52.2
1807	2827	3055	3075	51.8	47.6	5327	3207	3187	50.5	47.6	1.3	153	74	45.1	40	52
1808	2828	3055	3076	52.4	45.5	5328	3210	3190	50.5	47.6	2	156	74.2	45.5	40	52.2
1809	2829	24379	24398	55	55	5329	24582	24560	54.2	52.2	0.9	204	74.3	43.1	40	53.3
1810	2830	7876	7895	51.5	45	5330	8054	8035	50.4	50	1.1	179	73.5	42.5	40	51.7
1811	2831	3055	3076	52.4	45.5	5331	3207	3187	50.5	47.6	2	153	74	45.1	40	52
1812	2832	9130	9150	51.3	42.9	5332	9324	9300	52.9	40	1.6	195	73.9	42.6	40	52.2
1813	2833	9130	9150	51.3	42.9	5333	9324	9301	52.4	41.7	1.1	195	73.9	42.6	40	52.2
1814	2834	8794	8813	51.6	45	5334	9324	9301	52.4	41.7	0.8	531	75.7	41.6	40	53.6
1815	2835	8794	8813	51.6	45	5335	9324	9300	52.9	40	1.3	531	75.7	41.6	40	53.6
1816	2836	9130	9150	51.3	42.9	5336	9328	9310	51	52.6	0.3	199	74.2	43.2	40	52.4
1817	2837	9130	9150	51.3	42.9	5337	9333	9315	52.2	52.6	0.9	204	74.5	43.6	40	52.6
1818	2838	24179	24200	53.3	40.9	5338	24807	24786	51.7	45.5	1.6	629	75.8	41.3	40	53.7
1819	2839	4593	4613	51.5	47.6	5339	4708	4690	50.3	47.4	1.3	116	71.4	42.2	40	50.2
1820	2840	9130	9150	51.3	42.9	5340	9374	9353	50.1	40.9	1.2	245	74.6	42.4	40	52.3
1821	2841	29180	29199	50.1	40	5341	29412	29393	50.3	45	0.2	233	75.5	45.1	40	53
1822	2842	25348	25366	51.2	47.4	5342	25772	25753	51.9	50	0.8	425	74.9	40.5	40	52.9
1823	2843	24179	24200	53.3	40.9	5343	24815	24792	53.4	41.7	0.2	637	75.8	41.3	40	54.1
1824	2844	8794	8813	51.6	45	5344	9101	9081	50.5	47.6	1.2	308	74.8	41.6	40	52.6
1825	2845	16861	16880	50.8	50	5345	17056	17035	51.8	45.5	1	196	74.7	44.4	40	52.6
1826	2846	16562	16581	52.6	50	5346	17038	17021	50.7	50	1.9	477	75.7	41.9	40	53.3
1827	2847	16562	16581	52.6	50	5347	17039	17022	51.4	50	1.2	478	75.6	41.8	40	53.5
1828	2848	16562	16581	52.6	50	5348	17041	17023	53.5	52.6	0.9	480	75.7	41.9	40	53.8
1829	2849	3090	3110	50.3	42.9	5349	3647	3628	50.6	45	0.3	558	76.2	42.7	40	53.5
1830	2850	16562	16580	51.9	52.6	5350	17038	17021	50.7	50	1.2	477	75.7	41.9	40	53.3
1831	2851	16562	16580	51.9	52.6	5351	17039	17022	51.4	50	0.5	478	75.6	41.8	40	53.5
1832	2852	24178	24198	52.7	42.9	5352	24815	24792	53.4	41.7	0.7	638	75.7	41.2	40	53.9
1833	2853	16562	16580	51.9	52.6	5353	17041	17023	53.5	52.6	1.6	480	75.7	41.9	40	53.6
1834	2854	24179	24198	51	45	5354	24807	24786	51.7	45.5	0.7	629	75.8	41.3	40	53.5
1835	2855	24179	24198	51	45	5355	24818	24797	51.6	40.9	0.6	640	75.8	41.2	40	53.4
1836	2856	3090	3110	50.3	42.9	5356	3646	3625	52	40.9	1.7	557	76.1	42.5	40	53.5
1837	2857	3089	3110	51.8	45.5	5357	3650	3631	53.1	50	1.3	562	76.3	42.9	40	54
1838	2858	29259	29279	54	52.4	5358	29358	29339	52.8	50	1.1	100	72.4	47	40	51.6
1839	2859	8794	8813	51.6	45	5359	8928	8911	51.9	50	0.2	135	72.2	42.2	40	51.1
1840	2860	24176	24197	52.1	40.9	5360	24815	24792	53.4	41.7	1.3	640	75.8	41.2	40	53.8
1841	2861	29259	29277	50.9	52.6	5361	29358	29339	52.8	50	2	100	72.4	47	40	51.1
1842	2862	29257	29276	51.3	50	5362	29358	29339	52.8	50	1.5	102	72.6	47.1	40	51.3
1843	2863	9915	9935	51.8	47.6	5363	10017	9999	52.8	52.6	1	103	72.9	47.6	40	51.6
1844	2864	4639	4659	51.1	47.6	5364	5306	5288	52.4	52.6	1.3	668	75.6	40.9	40	53.4
1845	2865	24178	24197	50.3	40	5365	24807	24786	51.7	45.5	1.4	630	75.8	41.3	40	53.2
1846	2866	28653	28671	50.2	52.6	5366	29414	29395	50.5	50	0.3	762	78	46.2	40	54.7
1847	2867	28653	28671	50.2	52.6	5367	29412	29393	50.3	45	0.1	760	78	46.2	40	54.7
1848	2868	28652	28671	52.8	55	5368	29358	29339	52.8	50	0	707	78	46.4	40	55.5
1849	2869	15752	15772	50.8	47.6	5369	16213	16195	50.8	52.6	0	462	75.4	41.3	40	53.1
1850	2870	24178	24197	50.3	40	5370	24818	24797	51.6	40.9	1.4	641	75.7	41.2	40	53.2
1851	2871	19794	19814	51.7	47.6	5371	19909	19885	52.5	40	0.8	116	71.8	43.1	40	50.8
1852	2872	8866	8885	51.1	45	5372	9341	9322	51.1	50	0	476	75.6	41.8	40	53.4

1853	2873	15951	15973	52.1	43.5	5373	16175	16155	51.8	47.6	0.3	225	73.7	40.9	40	52.2
1854	2874	24174	24195	52.5	40.9	5374	24815	24791	54.5	40	2	642	75.8	41.3	40	53.9
1855	2875	8866	8885	51.1	45	5375	9340	9319	50.8	45.5	0.3	475	75.6	41.7	40	53.2
1856	2876	15951	15973	52.1	43.5	5376	16169	16151	51.3	52.6	0.8	219	73.5	40.6	40	51.9
1857	2877	15951	15974	53.3	41.7	5377	16175	16154	53.4	45.5	0.1	225	73.7	40.9	40	52.7
1858	2878	27437	27456	50.2	40	5378	27541	27521	51.7	47.6	1.5	105	71.8	44.8	40	50.4
1859	2879	15650	15674	52.9	40	5379	16210	16192	54.3	52.6	1.4	561	75.1	40.1	40	53.6
1860	2880	8866	8885	51.1	45	5380	9334	9316	51.3	52.6	0.2	469	75.7	42	40	53.4
1861	2881	8866	8885	51.1	45	5381	9310	9291	51.2	45	0.1	445	75.3	41.3	40	53.2
1862	2882	8866	8885	51.1	45	5382	9252	9234	51.4	52.6	0.3	387	75.1	41.3	40	53
1863	2883	3360	3379	50.7	45	5383	3494	3473	50.4	40.9	0.3	135	73.7	45.9	40	51.8
1864	2884	8866	8885	51.1	45	5384	9248	9229	50.1	45	1	383	75.1	41.3	40	52.7
1865	2885	18081	18099	51.2	52.6	5385	18697	18679	51.9	52.6	0.7	617	76.3	42.6	40	53.9
1866	2886	8865	8884	50.4	45	5386	9257	9238	50.5	45	0.1	393	75	41	40	52.7
1867	2887	18081	18099	51.2	52.6	5387	18239	18220	50	45	1.2	159	74	44.7	40	51.9
1868	2888	18081	18099	51.2	52.6	5388	18238	18219	50.3	45	0.9	158	74.1	44.9	40	52
1869	2889	28117	28135	50.6	52.6	5389	28505	28487	50.2	47.4	0.4	389	79.5	51.9	40	55.8
1870	2890	8866	8885	51.1	45	5390	9109	9087	50.5	43.5	0.6	244	73.9	41	40	52
1871	2891	9055	9079	52.8	40	5391	9724	9706	51.3	52.6	1.5	670	75.4	40.3	40	53.3
1872	2892	3403	3423	54.1	47.6	5392	3502	3478	55.8	48	1.7	100	71.6	45	40	51.5
1873	2893	28855	28874	52.9	50	5393	29306	29288	53.5	52.6	0.6	452	77.1	45.6	40	54.9
1874	2894	24173	24194	52.5	40.9	5394	24815	24792	53.4	41.7	0.9	643	75.8	41.2	40	53.9
1875	2895	3094	3113	50	50	5395	3647	3628	50.6	45	0.6	554	76.2	42.8	40	53.5
1876	2896	24174	24194	50.9	42.9	5396	24807	24786	51.7	45.5	0.8	634	75.8	41.3	40	53.4
1877	2897	28856	28875	52.2	50	5397	29306	29288	53.5	52.6	1.3	451	77.1	45.7	40	54.7
1878	2898	24174	24194	50.9	42.9	5398	24818	24797	51.6	40.9	0.7	645	75.8	41.2	40	53.4
1879	2899	28857	28876	51.7	45	5399	29306	29288	53.5	52.6	1.8	450	77.1	45.6	40	54.6
1880	2900	8858	8877	51.2	45	5400	9254	9236	50.6	47.4	0.6	397	75	41.1	40	52.8
1881	2901	16553	16571	53.4	52.6	5401	16777	16758	51.5	50	1.9	225	73.7	40.9	40	52.1
1882	2902	29197	29219	54.8	47.8	5402	29301	29282	55.3	55	0.5	105	73.4	48.6	40	52.9
1883	2903	29198	29219	52.6	45.5	5403	29306	29288	53.5	52.6	0.9	109	73.3	47.7	40	52.2
1884	2904	28857	28877	52.3	42.9	5404	29306	29288	53.5	52.6	1.2	450	77.1	45.6	40	54.8
1885	2905	29199	29219	51.2	42.9	5405	29298	29280	51.4	52.6	0.2	100	72.4	47	40	51.1
1886	2906	3094	3113	50	50	5406	3646	3625	52	40.9	2	553	76.2	42.7	40	53.4
1887	2907	3224	3243	52.3	50	5407	3650	3631	53.1	50	0.8	427	75.5	41.9	40	53.7
1888	2908	29195	29216	53.8	45.5	5408	29306	29287	54.6	55	0.8	112	73.6	48.2	40	52.8
1889	2909	28867	28885	51.5	52.6	5409	29358	29339	52.8	50	1.4	492	76.9	44.9	40	54.4
1890	2910	29196	29216	52.5	47.6	5410	29298	29279	52.6	55	0.1	103	73.3	48.5	40	52.1
1891	2911	28867	28886	53.2	50	5411	29415	29395	53.4	52.4	0.2	549	77.1	45	40	55
1892	2912	3093	3113	51.7	47.6	5412	3650	3631	53.1	50	1.4	558	76.3	42.8	40	54
1893	2913	3225	3243	50.9	52.6	5413	3646	3625	52	40.9	1.2	422	75.4	41.7	40	53.1
1894	2914	3225	3243	50.9	52.6	5414	3647	3628	50.6	45	0.3	423	75.5	41.8	40	53.1
1895	2915	28867	28886	53.2	50	5415	29306	29287	54.6	55	1.4	440	76.9	45.2	40	54.9
1896	2916	3223	3241	50.2	52.6	5416	3500	3481	51.2	50	1	278	74.7	42.1	40	52.5
1897	2917	28867	28886	53.2	50	5417	29298	29279	52.6	55	0.5	432	76.8	45.1	40	54.7
1898	2918	24034	24053	53.4	55	5418	24815	24791	54.5	40	1.1	782	76.3	42.1	40	54.5
1899	2919	3221	3239	51.5	52.6	5419	3650	3631	53.1	50	1.6	430	75.5	41.9	40	53.4
1900	2920	18080	18099	53	50	5420	18696	18673	53.4	41.7	0.5	617	76.2	42.5	40	54.3
1901	2921	3095	3116	51.9	45.5	5421	3650	3631	53.1	50	1.2	556	76.2	42.8	40	54
1902	2922	18080	18099	53	50	5422	18696	18672	53.9	40	1	617	76.2	42.5	40	54.3
1903	2923	28868	28887	50.7	45	5423	29298	29279	52.6	55	1.9	431	76.8	45	40	54.1

1904	2924	3218	3238	52.1	47.6	5424	3650	3631	53.1	50	1	433	75.5	41.8	40	53.6
1905	2925	8867	8886	50.7	50	5425	9252	9234	51.4	52.6	0.8	386	75.1	41.5	40	52.9
1906	2926	28867	28887	53.7	47.6	5426	29306	29287	54.6	55	0.8	440	76.9	45.2	40	55.1
1907	2927	3218	3237	50.5	45	5427	3497	3478	51.3	50	0.8	280	74.6	41.8	40	52.5
1908	2928	29195	29215	53.2	47.6	5428	29306	29287	54.6	55	1.4	112	73.6	48.2	40	52.6
1909	2929	29196	29215	51.8	50	5429	29298	29279	52.6	55	0.8	103	73.3	48.5	40	51.9
1910	2930	3218	3237	50.5	45	5430	3500	3481	51.2	50	0.6	283	74.6	41.7	40	52.5
1911	2931	8867	8886	50.7	50	5431	9245	9226	50	45	0.6	379	75	41.2	40	52.6
1912	2932	28868	28888	51.4	42.9	5432	29298	29279	52.6	55	1.2	431	76.8	45	40	54.3
1913	2933	8867	8886	50.7	50	5433	9107	9086	51.6	45.5	0.9	241	74.1	41.5	40	52.2
1914	2934	28867	28888	54.3	45.5	5434	29306	29287	54.6	55	0.3	440	76.9	45.2	40	55.2
1915	2935	19906	19925	50.1	50	5435	20615	20597	50.6	47.4	0.5	710	75.5	40.3	40	53
1916	2936	16551	16568	51.1	50	5436	16775	16756	50.3	45	0.8	225	73.8	41.3	40	51.9
1917	2937	8861	8880	50.2	45	5437	9341	9322	51.1	50	0.9	481	75.5	41.6	40	53
1918	2938	16368	16387	50.2	45	5438	16781	16761	51.3	47.6	1	414	75	40.8	40	52.7
1919	2939	3055	3074	51.1	50	5439	3209	3189	50.5	47.6	0.6	155	74.1	45.2	40	52.1
1920	2940	3217	3236	51.1	50	5440	3650	3631	53.1	50	2	434	75.5	41.9	40	53.3
1921	2941	28868	28889	52	40.9	5441	29298	29279	52.6	55	0.6	431	76.8	45	40	54.5
1922	2942	28867	28889	54.8	43.5	5442	29306	29287	54.6	55	0.2	440	76.9	45.2	40	55.3
1923	2943	3404	3422	50.5	47.4	5443	3503	3484	51.5	50	0.9	100	71.6	45	40	50.4
1924	2944	16368	16387	50.2	45	5444	16777	16758	51.5	50	1.2	410	75	40.7	40	52.6
1925	2945	24029	24047	52.1	52.6	5445	24815	24792	53.4	41.7	1.3	787	76.3	42.1	40	54.1
1926	2946	16368	16387	50.2	45	5446	16711	16691	51	42.9	0.8	344	75.1	41.9	40	52.7
1927	2947	28867	28890	55.2	41.7	5447	29306	29287	54.6	55	0.6	440	76.9	45.2	40	55.3
1928	2948	29196	29214	51.1	52.6	5448	29298	29279	52.6	55	1.5	103	73.3	48.5	40	51.7
1929	2949	18488	18507	53.7	55	5449	19224	19200	52.4	40	1.3	737	76	41.5	40	54
1930	2950	28395	28413	50.2	42.1	5450	28506	28488	50.2	47.4	0	112	74.4	50	40	52.2
1931	2951	16551	16568	51.1	50	5451	17032	17011	52	45.5	0.9	482	75.8	42.1	40	53.5
1932	2952	28871	28891	50.9	42.9	5452	29358	29339	52.8	50	1.9	488	76.9	44.9	40	54.2
1933	2953	28871	28891	50.9	42.9	5453	29298	29280	51.4	52.6	0.5	428	76.8	45.1	40	54.2
1934	2954	28870	28891	52.2	40.9	5454	29306	29288	53.5	52.6	1.2	437	76.8	45.1	40	54.6
1935	2955	28868	28891	53.8	41.7	5455	29301	29282	55.3	55	1.5	434	76.9	45.2	40	55.1
1936	2956	3404	3422	50.5	47.4	5456	3504	3485	50.4	45	0.1	101	71.5	44.6	40	50.3
1937	2957	29195	29213	51.9	52.6	5457	29298	29279	52.6	55	0.7	104	73.1	48.1	40	51.9
1938	2958	28938	28956	50.8	47.4	5458	29298	29280	51.4	52.6	0.6	361	76.4	44.9	40	53.8
1939	2959	18488	18507	53.7	55	5459	19210	19191	52	50	1.7	723	76	41.6	40	53.9
1940	2960	3095	3116	51.9	45.5	5460	3647	3628	50.6	45	1.3	553	76.2	42.7	40	53.6
1941	2961	3214	3233	51.1	50	5461	3497	3478	51.3	50	0.2	284	74.7	41.9	40	52.7
1942	2962	24017	24039	53	43.5	5462	24815	24791	54.5	40	1.5	799	76.2	41.9	40	54.4
1943	2963	3095	3116	51.9	45.5	5463	3646	3625	52	40.9	0.1	552	76.1	42.6	40	54
1944	2964	18550	18571	50.4	40.9	5464	19215	19194	50.2	40.9	0.2	666	75.8	41.1	40	53.2
1945	2965	3214	3233	51.1	50	5465	3500	3481	51.2	50	0.1	287	74.7	41.8	40	52.7
1946	2966	18586	18603	50.4	44.4	5466	19224	19200	52.4	40	1.9	639	75.6	40.8	40	53.1
1947	2967	18586	18603	50.4	44.4	5467	19217	19196	50.2	40.9	0.2	632	75.6	41	40	53.1
1948	2968	18586	18603	50.4	44.4	5468	19215	19194	50.2	40.9	0.2	630	75.6	41	40	53.1
1949	2969	18590	18608	50.6	42.1	5469	19224	19200	52.4	40	1.8	635	75.6	40.9	40	53.2
1950	2970	15255	15273	50.3	52.6	5470	15767	15746	50.7	40.9	0.4	513	75.1	40.2	40	52.7
1951	2971	28942	28961	50.2	45	5471	29414	29395	50.5	50	0.3	473	76.8	44.6	40	53.9
1952	2972	18590	18608	50.6	42.1	5472	19217	19196	50.2	40.9	0.3	628	75.7	41.1	40	53.1
1953	2973	3055	3074	51.1	50	5473	3207	3187	50.5	47.6	0.6	153	74	45.1	40	52
1954	2974	18590	18608	50.6	42.1	5474	19215	19194	50.2	40.9	0.3	626	75.7	41.1	40	53.1

1955	2975	18591	18611	51.7	42.9	5475	19224	19200	52.4	40	0.7	634	75.7	41	40	53.6
1956	2976	18591	18611	51.7	42.9	5476	19217	19196	50.2	40.9	1.4	627	75.7	41.1	40	53.2
1957	2977	18591	18611	51.7	42.9	5477	19215	19194	50.2	40.9	1.4	625	75.7	41.1	40	53.2
1958	2978	28546	28565	52.2	50	5478	28672	28654	50.6	52.6	1.6	127	76.5	53.5	40	53.8
1959	2979	29191	29210	54.4	55	5479	29415	29395	53.4	52.4	1	225	75.7	45.8	40	54.1
1960	2980	7880	7900	50.3	42.9	5480	8190	8172	50.3	47.4	0	311	74.9	41.8	40	52.6
1961	2981	3167	3189	51.6	43.5	5481	3650	3631	53.1	50	1.5	484	75.8	42.1	40	53.6
1962	2982	28965	28984	52.9	55	5482	29306	29288	53.5	52.6	0.6	342	76.5	45.3	40	54.5
1963	2983	3166	3188	51.6	43.5	5483	3650	3631	53.1	50	1.5	485	75.8	42.3	40	53.7
1964	2984	8867	8887	52.3	47.6	5484	9101	9081	50.5	47.6	1.9	235	74.1	41.7	40	52.1
1965	2985	23843	23863	50.3	42.9	5485	24013	23995	50.3	47.4	0	171	73.7	43.3	40	51.8
1966	2986	3403	3421	53.1	52.6	5486	3503	3484	51.5	50	1.7	101	71.9	45.5	40	50.9
1967	2987	16549	16567	54.9	52.6	5487	16777	16756	53.4	45.5	1.5	229	74	41.5	40	52.9
1968	2988	8868	8889	50.4	40.9	5488	9109	9087	50.5	43.5	0.1	242	73.9	40.9	40	51.9
1969	2989	8861	8880	50.2	45	5489	9311	9292	50.7	50	0.6	451	75.3	41.2	40	52.9
1970	2990	8868	8889	50.4	40.9	5490	9257	9238	50.5	45	0.1	390	75	41	40	52.7
1971	2991	8868	8889	50.4	40.9	5491	9313	9294	50.4	50	0	446	75.4	41.5	40	53
1972	2992	23841	23859	50.5	52.6	5492	24013	23995	50.3	47.4	0.1	173	74	43.9	40	52
1973	2993	28548	28568	50.5	42.9	5493	28672	28654	50.6	52.6	0	125	76.2	52.8	40	53.6
1974	2994	8867	8888	52.7	45.5	5494	9310	9291	51.2	45	1.5	444	75.4	41.4	40	53.2
1975	2995	28968	28988	50.9	47.6	5495	29298	29279	52.6	55	1.8	331	76.2	44.7	40	53.7
1976	2996	19907	19926	52.1	55	5496	20615	20597	50.6	47.4	1.6	709	75.5	40.3	40	53.1
1977	2997	8861	8880	50.2	45	5497	9252	9235	50.1	50	0.1	392	75	41.1	40	52.6
1978	2998	19909	19929	50.7	52.4	5498	20615	20597	50.6	47.4	0.2	707	75.5	40.3	40	53.1
1979	2999	3361	3382	51.9	45.5	5499	3500	3481	51.2	50	0.7	140	74.1	46.4	40	52.3
1980	3000	18696	18715	51.7	50	5500	18881	18862	50.2	45	1.5	186	74.1	43.5	40	52.1
1981	3001	28968	28989	51.5	45.5	5501	29298	29279	52.6	55	1.1	331	76.2	44.7	40	53.9
1982	3002	19709	19730	51.3	40.9	5502	19923	19903	50.9	47.6	0.4	215	73.9	41.9	40	52.1
1983	3003	3361	3382	51.9	45.5	5503	3497	3478	51.3	50	0.6	137	74.1	46.7	40	52.4
1984	3004	3361	3384	53.7	41.7	5504	3495	3473	51.8	43.5	1.9	135	74	46.7	40	52.5
1985	3005	19709	19730	51.3	40.9	5505	19924	19905	50.1	50	1.2	216	73.9	41.7	40	51.8
1986	3006	16378	16397	50.4	45	5506	16711	16691	51	42.9	0.6	334	75.2	42.2	40	52.9
1987	3007	3361	3382	51.9	45.5	5507	3504	3485	50.4	45	1.5	144	74.3	46.5	40	52.2
1988	3008	18704	18724	50.8	47.6	5508	19406	19388	50.6	47.4	0.1	703	75.4	40.3	40	53.1
1989	3009	8868	8889	50.4	40.9	5509	9314	9295	51.1	50	0.7	447	75.5	41.6	40	53
1990	3010	3361	3382	51.9	45.5	5510	3503	3484	51.5	50	0.5	143	74.4	46.9	40	52.6
1991	3011	19709	19730	51.3	40.9	5511	19931	19912	50.9	55	0.4	223	74.2	42.2	40	52.3
1992	3012	16548	16566	54.9	52.6	5512	16777	16756	53.4	45.5	1.5	230	73.9	41.3	40	52.9
1993	3013	8868	8889	50.4	40.9	5513	9315	9296	50	45	0.4	448	75.4	41.5	40	52.9
1994	3014	22321	22341	51.6	42.9	5514	22460	22441	50.7	45	0.9	140	71.5	40	40	50.3
1995	3015	29182	29202	51.2	42.9	5515	29412	29393	50.3	45	0.9	231	75.4	45	40	53
1996	3016	22173	22193	51	42.9	5516	22460	22441	50.7	45	0.3	288	74.1	40.3	40	52.1
1997	3017	29181	29201	52.4	47.6	5517	29413	29393	51.1	42.9	1.3	233	75.5	45.1	40	53.3
1998	3018	18704	18724	50.8	47.6	5518	18881	18862	50.2	45	0.5	178	73.8	43.3	40	51.9
1999	3019	20751	20771	51.3	47.6	5519	21301	21278	51.3	41.7	0	551	75.5	41	40	53.3
2000	3020	29181	29200	50	45	5520	29412	29393	50.3	45	0.3	232	75.5	45.3	40	53
2001	3021	20751	20771	51.3	47.6	5521	21304	21283	50.5	40.9	0.8	554	75.5	41	40	53.1
2002	3022	29173	29197	54.2	40	5522	29415	29395	53.4	52.4	0.8	243	75.7	45.3	40	54.1
2003	3023	8867	8888	52.7	45.5	5523	9247	9226	52	45.5	0.7	381	75	41.2	40	53.2
2004	3024	8867	8888	52.7	45.5	5524	9255	9236	51.1	45	1.6	389	75	41.1	40	52.9
2005	3025	29178	29198	51.4	42.9	5525	29412	29393	50.3	45	1.1	235	75.5	45.1	40	53.1

2006	3026	3163	3185	53.6	47.8	5526	3650	3631	53.1	50	0.5	488	75.9	42.4	40	54.2
2007	3027	19800	19817	50.4	50	5527	20033	20016	50.4	50	0	234	74.9	43.6	41	52.7
2008	3028	8867	8886	50.7	50	5528	9376	9355	51	40.9	0.3	510	75.7	41.8	41	53.3
2009	3029	19800	19817	50.4	50	5529	19930	19910	50.6	47.6	0.2	131	72.6	43.5	41	51
2010	3030	24418	24439	52.9	45.5	5530	25082	25064	51.1	52.6	1.8	665	75.8	41.2	41	53.5
2011	3031	25771	25790	51.1	45	5531	26182	26161	51.2	40.9	0.1	412	74.8	40.3	41	52.8
2012	3032	12976	12994	50.3	47.4	5532	13326	13306	50.7	42.9	0.3	351	76.1	44.2	41	53.5
2013	3033	12976	12994	50.3	47.4	5533	13328	13307	51.2	45.5	0.9	353	76.1	44.2	41	53.5
2014	3034	2823	2844	50.4	45.5	5534	3500	3481	51.2	50	0.7	678	76.3	42.5	41	53.7
2015	3035	18009	18028	51.6	55	5535	18223	18205	53.3	52.6	1.7	215	74.5	43.3	41	52.7
2016	3036	8223	8240	50.4	50	5536	8933	8916	52.2	50	1.8	711	75.4	40.1	41	53
2017	3037	29180	29199	50.1	40	5537	29414	29395	50.5	50	0.4	235	75.5	45.1	41	53
2018	3038	19800	19817	50.4	50	5538	19925	19906	50.1	50	0.4	126	72.4	43.7	41	50.8
2019	3039	25772	25793	52.4	40.9	5539	26183	26162	52.8	45.5	0.4	412	74.8	40.3	41	53.2
2020	3040	14951	14975	52.2	40	5540	15152	15135	51.4	50	0.8	202	73.4	41.1	41	51.9
2021	3041	2823	2844	50.4	45.5	5541	3503	3484	51.5	50	1	681	76.4	42.6	41	53.7
2022	3042	18075	18095	50.6	47.6	5542	18231	18210	52.2	45.5	1.6	157	73.6	43.9	41	51.8
2023	3043	5	23	51.3	52.6	5543	269	251	51.1	52.6	0.1	265	76.4	46.4	41	53.9
2024	3044	9140	9159	50.1	45	5544	9249	9231	50.8	47.4	0.7	110	71.3	42.7	41	50
2025	3045	24418	24439	52.9	45.5	5545	24815	24791	54.5	40	1.6	398	75.9	43.2	41	54.1
2026	3046	8794	8813	51.6	45	5546	9358	9338	51	42.9	0.6	565	75.8	41.8	41	53.5
2027	3047	24418	24439	52.9	45.5	5547	24807	24786	51.7	45.5	1.2	390	75.9	43.3	41	53.8
2028	3048	2387	2405	51.6	52.6	5548	3186	3165	50.4	40.9	1.2	800	76.9	43.5	41	54.1
2029	3049	24418	24439	52.9	45.5	5549	24527	24506	51.7	40.9	1.2	110	71.3	42.7	41	50.5
2030	3050	24418	24439	52.9	45.5	5550	24517	24494	53.2	41.7	0.3	100	70.8	43	41	50.5
2031	3051	4255	4276	51.7	45.5	5551	4836	4817	51.2	45	0.5	582	75.9	41.9	41	53.6
2032	3052	24420	24440	50.8	42.9	5552	25082	25064	51.1	52.6	0.3	663	75.7	41	41	53.3
2033	3053	8867	8887	52.3	47.6	5553	9250	9232	51.6	47.4	0.8	384	75.1	41.4	41	53.2
2034	3054	14951	14975	52.2	40	5554	15275	15257	50.8	52.6	1.3	325	74.6	40.9	41	52.6
2035	3055	2387	2405	51.6	52.6	5555	3185	3164	51	45.5	0.7	799	76.9	43.6	41	54.2
2036	3056	8865	8884	50.4	45	5556	9252	9235	50.1	50	0.3	388	75.1	41.2	41	52.7
2037	3057	24420	24440	50.8	42.9	5557	24818	24797	51.6	40.9	0.8	399	75.8	42.9	41	53.4
2038	3058	24420	24440	50.8	42.9	5558	24807	24786	51.7	45.5	0.9	388	75.8	43	41	53.4
2039	3059	11541	11560	50.1	45	5559	12110	12090	51.1	42.9	1	570	75.9	41.9	41	53.3
2040	3060	2387	2405	51.6	52.6	5560	2672	2653	51.6	50	0	286	77	47.6	41	54.5
2041	3061	24420	24440	50.8	42.9	5561	24526	24506	50.3	42.9	0.5	107	70.8	42.1	41	49.8
2042	3062	11540	11557	50.4	50	5562	12110	12090	51.1	42.9	0.7	571	76	42	41	53.4
2043	3063	6263	6282	50.9	45	5563	6483	6463	50.2	42.9	0.7	221	73.7	41.2	41	51.8
2044	3064	24418	24440	55	47.8	5564	24815	24791	54.5	40	0.5	398	75.9	43.2	41	54.6
2045	3065	18075	18095	50.6	47.6	5565	18233	18214	52	50	1.4	159	74	44.7	41	52.1
2046	3066	18075	18095	50.6	47.6	5566	18233	18215	51.3	52.6	0.7	159	74	44.7	41	52.1
2047	3067	2429	2447	50.2	47.4	5567	3055	3036	50.6	50	0.4	627	76.3	42.6	41	53.6
2048	3068	19800	19818	52.1	52.6	5568	19917	19896	50.9	45.5	1.2	118	71.9	43.2	41	50.7
2049	3069	24481	24500	50.1	45	5569	24936	24919	51.8	50	1.7	456	75.7	42.1	41	53.1
2050	3070	276	294	50.5	47.4	5570	713	695	50.7	47.4	0.2	438	79.1	50.7	41	55.7
2051	3071	19801	19819	53.2	52.6	5571	19927	19908	52.1	55	1.1	127	72.7	44.1	41	51.6
2052	3072	19801	19819	53.2	52.6	5572	19925	19905	51.4	52.4	1.9	125	72.5	44	41	51.3
2053	3073	3800	3824	53.6	40	5573	4318	4294	54.4	40	0.8	519	75.3	40.8	41	53.9
2054	3074	11540	11557	50.4	50	5574	12258	12238	50.3	42.9	0.2	719	76.2	42	41	53.5
2055	3075	24482	24502	50.3	42.9	5575	24938	24921	50.4	50	0.1	457	75.6	41.8	41	53.1
2056	3076	24482	24502	50.3	42.9	5576	24807	24786	51.7	45.5	1.4	326	75.4	42.9	41	53

2057	3077	8867	8888	52.7	45.5	5577	9364	9346	53.9	52.6	1.2	498	75.8	42.2	41	54
2058	3078	24481	24502	51.5	45.5	5578	25080	25062	53.5	52.6	2	600	75.5	40.8	41	53.4
2059	3079	8865	8884	50.4	45	5579	9107	9086	51.6	45.5	1.2	243	74	41.2	41	52
2060	3080	8867	8888	52.7	45.5	5580	9313	9293	52.1	47.6	0.6	447	75.5	41.6	41	53.5
2061	3081	2427	2445	52.1	52.6	5581	3055	3036	50.6	50	1.5	629	76.4	42.8	41	53.7
2062	3082	2823	2844	50.4	45.5	5582	3504	3485	50.4	45	0.1	682	76.3	42.5	41	53.7
2063	3083	24483	24503	51	42.9	5583	25085	25068	50.3	50	0.6	603	75.4	40.6	41	53
2064	3084	15255	15273	50.3	52.6	5584	15649	15632	50.1	50	0.2	395	75.1	41.3	41	52.7
2065	3085	24483	24503	51	42.9	5585	25082	25064	51.1	52.6	0.1	600	75.5	40.8	41	53.3
2066	3086	8867	8886	50.7	50	5586	9375	9354	50.4	40.9	0.3	509	75.7	41.8	41	53.2
2067	3087	12976	12994	50.3	47.4	5587	13329	13308	50.5	40.9	0.2	354	76.1	44.1	41	53.4
2068	3088	24483	24503	51	42.9	5588	25081	25063	52.4	52.6	1.4	599	75.5	40.7	41	53.2
2069	3089	379	398	50.1	45	5589	941	922	50.5	50	0.4	563	78.7	48.8	41	55.2
2070	3090	24483	24503	51	42.9	5590	24936	24919	51.8	50	0.8	454	75.7	42.1	41	53.4
2071	3091	19802	19820	53	52.6	5591	19927	19908	52.1	55	0.8	126	72.8	44.4	41	51.7
2072	3092	9934	9953	50.7	50	5592	10670	10649	51.3	40.9	0.6	737	75.7	40.8	41	53.3
2073	3093	8866	8885	51.1	45	5593	9312	9293	50.6	45	0.5	447	75.4	41.4	41	53
2074	3094	19846	19866	51.2	42.9	5594	20033	20016	50.4	50	0.8	188	74.2	43.6	41	52.2
2075	3095	19848	19867	50.7	45	5595	20033	20016	50.4	50	0.3	186	74.1	43.5	41	52.1
2076	3096	9538	9558	50.9	42.9	5596	10017	9999	52.8	52.6	1.9	480	75.5	41.5	41	53.2
2077	3097	8220	8238	51.5	47.4	5597	8933	8916	52.2	50	0.7	714	75.4	40.1	41	53.3
2078	3098	9140	9159	50.1	45	5598	9249	9232	50	50	0.1	110	71.3	42.7	41	50
2079	3099	12976	12994	50.3	47.4	5599	13332	13312	50.9	47.6	0.6	357	76.2	44.3	41	53.5
2080	3100	15752	15772	50.8	47.6	5600	16174	16154	50.4	42.9	0.4	423	75.1	40.9	41	52.8
2081	3101	18074	18094	51.1	42.9	5601	18232	18212	50.6	47.6	0.5	159	73.7	44	41	51.9
2082	3102	24559	24579	52	52.4	5602	25081	25063	52.4	52.6	0.4	523	75.5	41.1	41	53.5
2083	3103	24559	24579	52	52.4	5603	25079	25061	52.7	52.6	0.7	521	75.5	41.3	41	53.6
2084	3104	3169	3191	52.1	47.8	5604	3650	3631	53.1	50	1	482	75.9	42.3	41	53.8
2085	3105	28117	28135	50.6	52.6	5605	28672	28654	50.6	52.6	0.1	556	80	52	41	56.3
2086	3106	1809	1829	50.6	42.9	5606	2103	2082	52	45.5	1.5	295	75.4	43.4	41	53
2087	3107	24559	24579	52	52.4	5607	24933	24913	51.1	42.9	0.8	375	75.6	42.7	41	53.4
2088	3108	1809	1829	50.6	42.9	5608	2113	2094	50.1	45	0.4	305	75.4	43.3	41	52.9
2089	3109	28116	28134	50.8	47.4	5609	28505	28487	50.2	47.4	0.6	390	79.4	51.8	41	55.8
2090	3110	1808	1828	50.6	42.9	5610	2103	2082	52	45.5	1.5	296	75.5	43.6	41	53.1
2091	3111	15951	15975	53.1	40	5611	16210	16192	54.3	52.6	1.2	260	74.3	41.5	41	53.1
2092	3112	8865	8884	50.4	45	5612	9341	9322	51.1	50	0.7	477	75.6	41.7	41	53.1
2093	3113	15	33	50.7	52.6	5613	642	622	51.6	47.6	0.9	628	79	49.2	41	55.6
2094	3114	8861	8880	50.2	45	5614	9107	9086	51.6	45.5	1.4	247	73.9	40.9	41	51.9
2095	3115	1808	1828	50.6	42.9	5615	2113	2094	50.1	45	0.4	306	75.5	43.5	41	53
2096	3116	24562	24580	50.1	52.6	5616	24933	24913	51.1	42.9	1.1	372	75.5	42.5	41	53
2097	3117	28116	28134	50.8	47.4	5617	28672	28654	50.6	52.6	0.2	557	80	51.9	41	56.2
2098	3118	24560	24580	51.3	52.4	5618	25081	25063	52.4	52.6	1.1	522	75.4	41	41	53.3
2099	3119	24560	24580	51.3	52.4	5619	25079	25061	52.7	52.6	1.4	520	75.5	41.2	41	53.3
2100	3120	16366	16384	50.3	52.6	5620	16775	16755	51.1	42.9	0.7	410	75.1	41	41	52.7
2101	3121	16366	16384	50.3	52.6	5621	16774	16754	50.4	42.9	0.1	409	75.1	41.1	41	52.8
2102	3122	24569	24590	56.6	54.5	5622	25089	25070	55.8	55	0.8	521	75.4	40.9	41	54.6
2103	3123	24569	24590	56.6	54.5	5623	25088	25069	55	50	1.6	520	75.3	40.8	41	54.3
2104	3124	24567	24590	57.8	54.2	5624	25095	25072	59.3	54.2	1.5	529	75.4	40.8	41	55.2
2105	3125	24568	24591	58.9	54.2	5625	25095	25072	59.3	54.2	0.4	528	75.3	40.7	41	55.5
2106	3126	24568	24591	58.9	54.2	5626	25091	25070	59.1	54.5	0.2	524	75.4	40.8	41	55.5
2107	3127	24568	24591	58.9	54.2	5627	25090	25069	58.3	54.5	0.6	523	75.4	40.9	41	55.4

2108	3128	16366	16384	50.3	52.6	5628	16774	16753	51.1	40.9	0.8	409	75.1	41.1	41	52.8
2109	3129	1806	1825	51.1	45	5629	2103	2082	52	45.5	1	298	75.5	43.6	41	53.3
2110	3130	8374	8395	52.4	45.5	5630	9107	9086	51.6	45.5	0.8	734	75.5	40.2	41	53.4
2111	3131	24622	24643	57.1	54.5	5631	24935	24913	56.1	47.8	1	314	74.5	40.8	41	54.1
2112	3132	9130	9150	51.3	42.9	5632	9358	9338	51	42.9	0.3	229	74.5	42.8	41	52.5
2113	3133	12936	12957	53.7	45.5	5633	13530	13511	55.6	55	1.9	595	77.4	45.4	41	55.4
2114	3134	8373	8391	50.7	47.4	5634	9107	9086	51.6	45.5	0.9	735	75.4	40.1	41	53.1
2115	3135	1352	1371	56.1	55	5635	1701	1678	54.3	41.7	1.8	350	76.7	45.7	41	55.1
2116	3136	8867	8886	50.7	50	5636	9342	9323	52.1	50	1.4	476	75.7	42	41	53.3
2117	3137	1352	1371	56.1	55	5637	1701	1677	54.7	40	1.4	350	76.7	45.7	41	55.2
2118	3138	9130	9150	51.3	42.9	5638	9249	9232	50	50	1.3	120	71.7	42.5	41	50.3
2119	3139	16861	16880	50.8	50	5639	17062	17045	50.2	50	0.6	202	74.8	44.6	41	52.5
2120	3140	9130	9150	51.3	42.9	5640	9249	9231	50.8	47.4	0.5	120	71.7	42.5	41	50.5
2121	3141	9130	9150	51.3	42.9	5641	9249	9230	51.5	45	0.2	120	71.7	42.5	41	50.7
2122	3142	8372	8390	50.7	47.4	5642	9060	9039	50.3	40.9	0.4	689	75.4	40.2	41	53
2123	3143	18074	18093	50.3	45	5643	18232	18212	50.6	47.6	0.3	159	73.7	44	41	51.8
2124	3144	2671	2692	52.1	40.9	5644	3193	3172	52.6	50	0.5	523	75.8	41.9	41	53.8
2125	3145	16562	16581	52.6	50	5645	17064	17045	51.4	50	1.2	503	75.8	42.1	41	53.6
2126	3146	2671	2692	52.1	40.9	5646	3193	3173	51.4	47.6	0.7	523	75.8	41.9	41	53.6
2127	3147	8372	8390	50.7	47.4	5647	9107	9086	51.6	45.5	0.9	736	75.5	40.2	41	53.1
2128	3148	12726	12746	51.3	47.6	5648	13321	13301	50.3	42.9	0.9	596	76.7	43.6	41	53.9
2129	3149	8867	8886	50.7	50	5649	9312	9293	50.6	45	0.1	446	75.4	41.5	41	53
2130	3150	16562	16580	51.9	52.6	5650	17062	17045	50.2	50	1.7	501	75.8	42.1	41	53.2
2131	3151	27377	27397	53.4	47.6	5651	27674	27653	52.5	40.9	0.9	298	74.3	40.6	41	52.8
2132	3152	16556	16573	50.3	50	5652	17111	17090	51.1	40.9	0.8	556	76.1	42.4	41	53.5
2133	3153	7815	7833	51.5	52.6	5653	8531	8512	52	45	0.5	717	75.7	40.7	41	53.5
2134	3154	3223	3241	50.2	52.6	5654	3494	3473	50.4	40.9	0.2	272	74.6	41.9	41	52.4
2135	3155	8372	8390	50.7	47.4	5655	9109	9087	50.5	43.5	0.1	738	75.4	40.1	41	53.1
2136	3156	3041	3065	57.7	48	5656	3650	3628	56.3	47.8	1.4	610	76.3	42.8	41	55.4
2137	3157	9569	9591	53	43.5	5657	10017	9999	52.8	52.6	0.3	449	75.4	41.4	41	53.7
2138	3158	3041	3065	57.7	48	5658	3649	3625	56.6	44	1.2	609	76.3	42.7	41	55.5
2139	3159	13176	13196	51.4	47.6	5659	13321	13301	50.3	42.9	1	146	73.3	43.8	41	51.5
2140	3160	16366	16385	52.9	55	5660	16775	16755	51.1	42.9	1.8	410	75.1	41	41	53
2141	3161	16366	16385	52.9	55	5661	16775	16754	51.7	40.9	1.1	410	75.1	41	41	53.2
2142	3162	16366	16385	52.9	55	5662	16774	16753	51.1	40.9	1.8	409	75.1	41.1	41	53
2143	3163	1402	1422	50.2	42.9	5663	2104	2084	50.6	42.9	0.4	703	76.7	43.2	41	53.8
2144	3164	1402	1422	50.2	42.9	5664	1697	1678	50.3	45	0.1	296	76	44.9	41	53.4
2145	3165	3055	3076	52.4	45.5	5665	3503	3484	51.5	50	1	449	76.1	43.2	41	53.8
2146	3166	15211	15230	50.2	45	5666	16001	15980	51.1	45.5	0.9	791	75.6	40.3	41	53.1
2147	3167	12267	12290	54.5	41.7	5667	12414	12392	53.9	43.5	0.6	148	72.2	41.2	41	51.8
2148	3168	8861	8880	50.2	45	5668	9256	9237	50.8	45	0.6	396	75	40.9	41	52.6
2149	3169	3049	3071	56.3	52.2	5669	3650	3628	56.3	47.8	0	602	76.4	42.9	41	55.4
2150	3170	3049	3071	56.3	52.2	5670	3648	3625	55.5	41.7	0.8	600	76.3	42.7	41	55.2
2151	3171	8861	8880	50.2	45	5671	9313	9294	50.4	50	0.3	453	75.3	41.3	41	52.9
2152	3172	12352	12375	52.9	41.7	5672	12911	12891	51.2	47.6	1.7	560	76.1	42.5	41	53.7
2153	3173	7965	7985	51.9	42.9	5673	8531	8512	52	45	0.2	567	75.1	40	41	53.2
2154	3174	18017	18036	54.8	55	5674	18233	18212	53.5	50	1.3	217	74.7	43.8	41	53.5
2155	3175	8867	8886	50.7	50	5675	9257	9238	50.5	45	0.2	391	75.1	41.2	41	52.8
2156	3176	3221	3239	51.5	52.6	5676	3494	3473	50.4	40.9	1.1	274	74.5	41.6	41	52.4
2157	3177	1402	1422	50.2	42.9	5677	1697	1677	51	42.9	0.8	296	76	44.9	41	53.4
2158	3178	1402	1422	50.2	42.9	5678	1697	1676	51.7	40.9	1.5	296	76	44.9	41	53.4
2159	3179	18011	18032	55.7	54.5	5679	18220	18201	56.1	55	0.4	210	74.5	43.3	41	53.9

2160	3180	12726	12746	51.3	47.6	5680	13329	13308	50.5	40.9	0.8	604	76.6	43.5	41	53.9
2161	3181	1402	1425	52.8	41.7	5681	1698	1678	51.7	42.9	1.1	297	76	44.8	41	53.8
2162	3182	18013	18032	52.2	55	5682	18223	18205	53.3	52.6	1.1	211	74.4	43.1	41	52.8
2163	3183	3777	3797	51.7	47.6	5683	4444	4424	50.6	42.9	1.1	668	75.6	40.7	41	53.2
2164	3184	3777	3797	51.7	47.6	5684	4445	4425	50.6	42.9	1.1	669	75.6	40.7	41	53.2
2165	3185	7876	7895	51.5	45	5685	8189	8170	50.6	50	0.9	314	75.1	42.4	41	52.9
2166	3186	18014	18032	51	52.6	5686	18229	18209	50.1	42.9	0.8	216	74.2	42.6	41	52.1
2167	3187	1402	1425	52.8	41.7	5687	1697	1677	51	42.9	1.8	296	76	44.9	41	53.6
2168	3188	1402	1425	52.8	41.7	5688	1697	1676	51.7	40.9	1.1	296	76	44.9	41	53.8
2169	3189	1402	1425	52.8	41.7	5689	1501	1481	51.2	42.9	1.6	100	72	46	41	50.9
2170	3190	12366	12384	51.7	52.6	5690	13155	13138	50.4	50	1.3	790	76.8	43.4	41	54
2171	3191	27361	27380	52.4	55	5691	27573	27552	52.3	40.9	0.1	213	75	44.6	41	53.3
2172	3192	18006	18028	54.5	52.2	5692	18220	18201	56.1	55	1.6	215	74.5	43.3	41	53.6
2173	3193	1442	1461	51.6	55	5693	1872	1854	53.2	52.6	1.6	431	76.2	43.6	41	53.9
2174	3194	27361	27380	52.4	55	5694	27567	27547	51.1	42.9	1.3	207	75.1	44.9	41	53
2175	3195	9131	9151	50.4	42.9	5695	9249	9230	51.5	45	1.2	119	71.8	42.9	41	50.5
2176	3196	3217	3236	51.1	50	5696	3504	3485	50.4	45	0.7	288	74.8	42	41	52.6
2177	3197	18011	18029	51.3	52.6	5697	18232	18212	50.6	47.6	0.7	222	74.8	43.7	41	52.6
2178	3198	3055	3074	51.1	50	5698	3503	3484	51.5	50	0.4	449	76.1	43.2	41	53.7
2179	3199	8866	8886	52.3	47.6	5699	9364	9346	53.9	52.6	1.6	499	75.8	42.1	41	53.9
2180	3200	16368	16387	50.2	45	5700	16774	16752	52.2	43.5	2	407	75	40.8	41	52.7
2181	3201	8859	8879	50	42.9	5701	9252	9235	50.1	50	0.1	394	75	41.1	41	52.6
2182	3202	9131	9151	50.4	42.9	5702	9249	9231	50.8	47.4	0.5	119	71.8	42.9	41	50.5
2183	3203	3217	3236	51.1	50	5703	3494	3473	50.4	40.9	0.7	278	74.6	41.7	41	52.4
2184	3204	8859	8879	50	42.9	5704	9341	9322	51.1	50	1.1	483	75.6	41.6	41	53
2185	3205	9131	9151	50.4	42.9	5705	9249	9232	50	50	0.3	119	71.8	42.9	41	50.4
2186	3206	8867	8886	50.7	50	5706	9248	9229	50.1	45	0.5	382	75.1	41.4	41	52.7
2187	3207	27366	27384	52.2	52.6	5707	27576	27555	51	40.9	1.2	211	74.8	44.1	41	52.7
2188	3208	1442	1461	51.6	55	5708	1879	1861	53	52.6	1.4	438	76.2	43.6	41	54
2189	3209	12366	12384	51.7	52.6	5709	12724	12705	52.4	55	0.7	359	75.6	42.9	41	53.5
2190	3210	12366	12384	51.7	52.6	5710	12498	12480	50	47.4	1.6	133	73	44.4	41	51.2
2191	3211	98	118	50.6	42.9	5711	713	695	50.7	47.4	0.1	616	79	49.4	41	55.6
2192	3212	12373	12391	50.8	47.4	5712	13155	13138	50.4	50	0.4	783	76.8	43.4	41	54
2193	3213	18011	18030	52.9	55	5713	18230	18209	51.3	45.5	1.6	220	74.5	43.2	41	52.7
2194	3214	1402	1426	54.1	40	5714	1700	1676	53.9	40	0.2	299	76	44.8	41	54.5
2195	3215	18011	18030	52.9	55	5715	18223	18205	53.3	52.6	0.5	213	74.4	43.2	41	53.1
2196	3216	1402	1426	54.1	40	5716	1698	1677	52.3	40.9	1.8	297	76	44.8	41	54
2197	3217	16463	16483	51.3	42.9	5717	17032	17011	52	45.5	0.7	570	76	42.1	41	53.7
2198	3218	18009	18030	54.6	54.5	5718	18220	18201	56.1	55	1.6	212	74.5	43.4	41	53.6
2199	3219	1402	1426	54.1	40	5719	1700	1678	52.9	43.5	1.3	299	76	44.8	41	54.2
2200	3220	9131	9151	50.4	42.9	5720	9358	9338	51	42.9	0.6	228	74.6	43	41	52.4
2201	3221	3055	3075	51.8	47.6	5721	3503	3484	51.5	50	0.3	449	76.1	43.2	41	53.8
2202	3222	18013	18031	50.6	52.6	5722	18232	18212	50.6	47.6	0	220	74.7	43.6	41	52.6
2203	3223	8794	8813	51.6	45	5723	9249	9230	51.5	45	0.1	456	75.4	41.4	41	53.4
2204	3224	8794	8813	51.6	45	5724	9249	9231	50.8	47.4	0.8	456	75.4	41.4	41	53.1
2205	3225	16549	16567	54.9	52.6	5725	17065	17045	53.1	47.6	1.9	517	76	42.4	41	54.2
2206	3226	8794	8813	51.6	45	5726	9249	9232	50	50	1.6	456	75.4	41.4	41	52.9
2207	3227	1402	1426	54.1	40	5727	2104	2082	53.5	43.5	0.6	703	76.7	43.2	41	54.8
2208	3228	9927	9946	51.3	50	5728	10356	10336	52.4	47.6	1.1	430	75.6	42.1	41	53.4
2209	3229	3219	3238	50.7	50	5729	3494	3473	50.4	40.9	0.3	276	74.5	41.7	41	52.4
2210	3230	16549	16567	54.9	52.6	5730	17033	17011	53.2	43.5	1.7	485	75.8	42.1	41	54.1

2211	3231	18014	18032	51	52.6	5731	18702	18685	50.2	50	0.8	689	76.2	42.2	41	53.5
2212	3232	8794	8813	51.6	45	5732	9333	9315	52.2	52.6	0.6	540	75.9	42	41	53.7
2213	3233	8867	8888	52.7	45.5	5733	9249	9229	53	47.6	0.2	383	75.2	41.5	41	53.5
2214	3234	18009	18028	51.6	55	5734	18229	18209	50.1	42.9	1.5	221	74.5	43	41	52.3
2215	3235	9633	9651	51	47.4	5735	10017	9999	52.8	52.6	1.8	385	75.6	42.6	41	53.3
2216	3236	9915	9935	51.8	47.6	5736	10449	10428	51.9	40.9	0.1	535	75.4	40.9	41	53.4
2217	3237	29259	29277	50.9	52.6	5737	29414	29395	50.5	50	0.3	156	74.5	46.2	41	52.4
2218	3238	8868	8889	50.4	40.9	5738	9317	9297	50.5	42.9	0.1	450	75.4	41.6	41	53
2219	3239	29257	29276	51.3	50	5739	29414	29395	50.5	50	0.8	158	74.6	46.2	41	52.5
2220	3240	13176	13196	51.4	47.6	5740	13332	13312	50.9	47.6	0.5	157	73.6	43.9	41	51.9
2221	3241	9918	9938	51.4	47.6	5741	10449	10428	51.9	40.9	0.5	532	75.4	40.8	41	53.3
2222	3242	13176	13196	51.4	47.6	5742	13856	13835	50.1	45.5	1.3	681	75.8	41.1	41	53.2
2223	3243	29253	29270	50	50	5743	29414	29395	50.5	50	0.5	162	75.2	47.5	41	52.8
2224	3244	13037	13058	54.8	50	5744	13530	13511	55.6	55	0.8	494	77.3	45.7	41	55.6
2225	3245	18009	18028	51.6	55	5745	18702	18685	50.2	50	1.5	694	76.3	42.4	41	53.6
2226	3246	24178	24197	50.3	40	5746	24938	24921	50.4	50	0.1	761	75.8	40.9	41	53.2
2227	3247	24174	24195	52.5	40.9	5747	24740	24717	52.5	41.7	0	567	76	42.2	41	54
2228	3248	7679	7698	50.6	50	5748	8054	8035	50.4	50	0.1	376	75.6	42.6	41	53.1
2229	3249	18005	18024	51.1	50	5749	18229	18209	50.1	42.9	1	225	74.4	42.7	41	52.2
2230	3250	24174	24195	52.5	40.9	5750	24933	24913	51.1	42.9	1.4	760	75.8	40.9	41	53.5
2231	3251	3016	3036	50.2	42.9	5751	3500	3481	51.2	50	0.9	485	76.3	43.3	41	53.6
2232	3252	28820	28838	53.7	52.6	5752	29306	29288	53.5	52.6	0.2	487	77.1	45.4	41	55.1
2233	3253	18005	18024	51.1	50	5753	18233	18214	52	50	0.9	229	74.9	43.7	41	52.8
2234	3254	3016	3036	50.2	42.9	5754	3503	3484	51.5	50	1.2	488	76.3	43.4	41	53.6
2235	3255	7723	7741	52.2	52.6	5755	8054	8035	50.4	50	1.7	332	75	41.9	41	52.8
2236	3256	29200	29224	54.2	40	5756	29358	29339	52.8	50	1.4	159	74.5	45.9	41	53.1
2237	3257	3016	3036	50.2	42.9	5757	3504	3485	50.4	45	0.1	489	76.3	43.4	41	53.6
2238	3258	985	1004	51.1	50	5758	1499	1482	50.1	50	1.1	515	76.5	43.7	41	53.7
2239	3259	8866	8885	51.1	45	5759	9257	9238	50.5	45	0.6	392	75	41.1	41	52.8
2240	3260	3016	3036	50.2	42.9	5760	3647	3628	50.6	45	0.4	632	76.4	42.9	41	53.7
2241	3261	13039	13058	51.8	50	5761	13749	13727	50.5	43.5	1.3	711	76.7	43.2	41	53.9
2242	3262	24096	24119	54.4	41.7	5762	24815	24792	53.4	41.7	1	720	75.8	41	41	54.2
2243	3263	17840	17859	50.8	45	5763	18229	18209	50.1	42.9	0.7	390	74.7	40.3	41	52.4
2244	3264	15255	15273	50.3	52.6	5764	15647	15628	51	45	0.7	393	75.1	41.2	41	52.7
2245	3265	988	1006	52.2	52.6	5765	1500	1482	50.6	47.4	1.6	513	76.5	43.7	41	53.8
2246	3266	24035	24053	52.2	52.6	5766	24527	24508	50.5	45	1.7	493	75.4	41.2	41	53
2247	3267	18616	18636	51.4	47.6	5767	19215	19194	50.2	40.9	1.1	600	75.7	41.2	41	53.1
2248	3268	8374	8393	51.2	45	5768	9101	9081	50.5	47.6	0.7	728	75.5	40.2	41	53.1
2249	3269	17840	17859	50.8	45	5769	18238	18219	50.3	45	0.5	399	75	40.9	41	52.7
2250	3270	24030	24047	50.7	50	5770	24526	24506	50.3	42.9	0.4	497	75.5	41.2	41	53
2251	3271	24030	24047	50.7	50	5771	24527	24507	51	42.9	0.3	498	75.4	41.2	41	53.1
2252	3272	17840	17859	50.8	45	5772	18239	18220	50	45	0.8	400	74.9	40.8	41	52.6
2253	3273	985	1008	56.1	50	5773	1626	1602	56.1	44	0	642	77.1	44.5	41	55.9
2254	3274	13039	13057	51.1	52.6	5774	13749	13727	50.5	43.5	0.6	711	76.7	43.2	41	53.9
2255	3275	29200	29223	53.7	41.7	5775	29358	29339	52.8	50	0.9	159	74.5	45.9	41	53.1
2256	3276	1046	1063	50.3	50	5776	1498	1481	51	50	0.7	453	76.4	43.9	41	53.7
2257	3277	24019	24039	50.1	42.9	5777	24527	24508	50.5	45	0.4	509	75.4	41.1	41	52.9
2258	3278	24014	24035	50.6	40.9	5778	24527	24508	50.5	45	0.1	514	75.5	41.2	41	53.1
2259	3279	1046	1063	50.3	50	5779	1497	1480	50.3	50	0.1	452	76.5	44	41	53.7
2260	3280	29201	29222	51	40.9	5780	29358	29339	52.8	50	1.9	158	74.3	45.6	41	52.4
2261	3281	18583	18603	54.8	47.6	5781	18696	18672	53.9	40	0.8	114	70.5	40.4	41	50.6

2262	3282	12977	12996	50.2	40	5782	13326	13306	50.7	42.9	0.4	350	76	44	41	53.4
2263	3283	23843	23863	50.3	42.9	5783	24088	24070	50.5	52.6	0.2	246	75.7	45.1	41	53.2
2264	3284	29200	29221	52.6	45.5	5784	29358	29339	52.8	50	0.2	159	74.5	45.9	41	53
2265	3285	23843	23863	50.3	42.9	5785	24091	24073	50.9	52.6	0.5	249	75.8	45.4	41	53.3
2266	3286	17792	17813	51.6	40.9	5786	18231	18210	52.2	45.5	0.6	440	75	40.5	41	53.1
2267	3287	23843	23863	50.3	42.9	5787	24094	24076	50.9	52.6	0.5	252	75.9	45.6	41	53.4
2268	3288	8374	8393	51.2	45	5788	9109	9087	50.5	43.5	0.6	736	75.4	40.1	41	53.1
2269	3289	17793	17813	50	42.9	5789	18223	18206	51.8	50	1.7	431	74.9	40.4	41	52.5
2270	3290	1046	1063	50.3	50	5790	1481	1463	50.5	47.4	0.2	436	76.2	43.6	41	53.6
2271	3291	23842	23862	50.9	47.6	5791	24093	24075	50.9	52.6	0	252	75.9	45.6	41	53.5
2272	3292	23842	23862	50.9	47.6	5792	24527	24507	51	42.9	0.1	686	76.1	41.8	41	53.6
2273	3293	2823	2844	50.4	45.5	5793	3082	3058	52.3	40	1.9	260	74.3	41.5	41	52.3
2274	3294	18550	18571	50.4	40.9	5794	19316	19295	50	40.9	0.4	767	75.5	40.3	41	53
2275	3295	23841	23860	52.1	55	5795	24527	24507	51	42.9	1.1	687	76.1	41.9	41	53.7
2276	3296	23841	23860	52.1	55	5796	24527	24508	50.5	45	1.6	687	76.1	41.9	41	53.5
2277	3297	17793	17813	50	42.9	5797	18233	18215	51.3	52.6	1.3	441	75.1	40.8	41	52.7
2278	3298	1	19	50.1	52.6	5798	269	251	51.1	52.6	1.1	269	76.4	46.5	41	53.6
2279	3299	23841	23859	50.5	52.6	5799	24094	24076	50.9	52.6	0.4	254	76.1	46.1	41	53.5
2280	3300	8908	8925	51.1	50	5800	9249	9231	50.8	47.4	0.2	342	75.1	41.8	41	52.9
2281	3301	8908	8925	51.1	50	5801	9249	9230	51.5	45	0.5	342	75.1	41.8	41	53
2282	3302	23841	23859	50.5	52.6	5802	24500	24481	50.1	45	0.4	660	76.1	42.1	41	53.4
2283	3303	23841	23859	50.5	52.6	5803	24526	24506	50.3	42.9	0.2	686	76.1	42	41	53.5
2284	3304	18225	18243	51.4	52.6	5804	18632	18611	50.2	40.9	1.2	408	75.7	42.6	41	53.2
2285	3305	3794	3812	52.9	52.6	5805	4318	4294	54.4	40	1.5	525	75.5	41.1	41	53.8
2286	3306	8908	8925	51.1	50	5806	9245	9226	50	45	1	338	74.9	41.4	41	52.5
2287	3307	17790	17811	51.6	40.9	5807	18231	18210	52.2	45.5	0.6	442	75	40.5	41	53.1
2288	3308	18077	18100	54.7	45.8	5808	18443	18424	55.9	55	1.3	367	75.8	43.3	41	54.6
2289	3309	23838	23857	50.4	50	5809	24527	24507	51	42.9	0.6	690	76	41.7	41	53.4
2290	3310	23838	23857	50.4	50	5810	24527	24508	50.5	45	0.1	690	76	41.7	41	53.4
2291	3311	23735	23752	51.2	50	5811	24013	23995	50.3	47.4	0.8	279	74.1	40.5	41	52.1
2292	3312	18080	18100	53.3	47.6	5812	18220	18202	54.8	52.6	1.5	141	73.1	44	41	52.3
2293	3313	18081	18100	51.7	50	5813	18223	18206	51.8	50	0.1	143	73.2	44.1	41	51.9
2294	3314	18081	18100	51.7	50	5814	18231	18210	52.2	45.5	0.5	151	73.6	44.4	41	52.1
2295	3315	18081	18100	51.7	50	5815	18233	18214	52	50	0.4	153	74	45.1	41	52.4
2296	3316	18081	18100	51.7	50	5816	18233	18215	51.3	52.6	0.4	153	74	45.1	41	52.3
2297	3317	8911	8928	51.9	50	5817	9252	9235	50.1	50	1.8	342	75	41.5	41	52.6
2298	3318	17791	17811	50	42.9	5818	18223	18206	51.8	50	1.7	433	74.9	40.4	41	52.5
2299	3319	8911	8928	51.9	50	5819	9249	9231	50.8	47.4	1	339	75	41.6	41	52.8
2300	3320	12352	12375	52.9	41.7	5820	12912	12892	53.6	52.4	0.8	561	76.2	42.6	41	54.3
2301	3321	8911	8928	51.9	50	5821	9249	9230	51.5	45	0.3	339	75	41.6	41	53
2302	3322	12352	12375	52.9	41.7	5822	12995	12976	51.1	45	1.8	644	76.4	42.9	41	53.9
2303	3323	17791	17811	50	42.9	5823	18233	18215	51.3	52.6	1.3	443	75.1	40.9	41	52.7
2304	3324	12977	12996	50.2	40	5824	13328	13307	51.2	45.5	1	352	76	44	41	53.4
2305	3325	12977	12996	50.2	40	5825	13329	13308	50.5	40.9	0.3	353	76	43.9	41	53.4
2306	3326	8911	8928	51.9	50	5826	9245	9226	50	45	1.8	335	74.8	41.2	41	52.5
2307	3327	8913	8931	55.5	52.6	5827	9252	9231	54	45.5	1.5	340	74.9	41.5	41	53.7
2308	3328	1402	1425	52.8	41.7	5828	1501	1480	51.9	40.9	0.9	100	72	46	41	51.1
2309	3329	24941	24960	52	50	5829	25646	25627	50.5	45	1.5	706	75.4	40.2	41	53
2310	3330	17608	17628	50.9	42.9	5830	17769	17749	50	42.9	0.9	162	72.4	40.7	41	50.8
2311	3331	24941	24960	52	50	5831	25404	25386	52.7	52.6	0.7	464	75.3	41.2	41	53.4
2312	3332	24941	24960	52	50	5832	25401	25383	50.6	47.4	1.4	461	75.2	41	41	53

2313	3333	24941	24960	52	50	5833	25400	25382	51.4	52.6	0.6	460	75.3	41.1	41	53.2
2314	3334	17608	17628	50.9	42.9	5834	18231	18210	52.2	45.5	1.2	624	75.2	40.1	41	53.1
2315	3335	18081	18099	51.2	52.6	5835	18232	18212	50.6	47.6	0.6	152	73.8	44.7	41	51.9
2316	3336	7725	7742	50	50	5836	7853	7833	50.7	47.6	0.7	129	71.2	40.3	41	49.9
2317	3337	8913	8931	55.5	52.6	5837	9252	9230	54.5	43.5	1	340	74.9	41.5	41	53.9
2318	3338	17608	17628	50.9	42.9	5838	18233	18215	51.3	52.6	0.4	626	75.3	40.3	41	53.1
2319	3339	19715	19735	52.5	47.6	5839	19931	19912	50.9	55	1.6	217	74	41.9	41	52.2
2320	3340	8913	8931	55.5	52.6	5840	9248	9226	54.7	47.8	0.7	336	74.9	41.4	41	53.9
2321	3341	18081	18099	51.2	52.6	5841	18642	18622	50.5	42.9	0.7	562	76.2	42.7	41	53.6
2322	3342	2823	2844	50.4	45.5	5842	3189	3168	51	45.5	0.5	367	75.6	42.8	41	53.2
2323	3343	19715	19735	52.5	47.6	5843	19927	19908	52.1	55	0.3	213	73.9	41.8	41	52.4
2324	3344	2823	2844	50.4	45.5	5844	3190	3169	50.7	45.5	0.2	368	75.6	42.7	41	53.1
2325	3345	28936	28956	55.2	52.4	5845	29306	29287	54.6	55	0.6	371	76.6	45.3	41	55.1
2326	3346	28936	28956	55.2	52.4	5846	29306	29285	56.7	54.5	1.6	371	76.6	45.3	41	55.3
2327	3347	28523	28544	51.6	40.9	5847	29298	29280	51.4	52.6	0.2	776	78.4	47.3	41	55.4
2328	3348	24180	24199	50.3	40	5848	24933	24913	51.1	42.9	0.9	754	75.8	40.8	41	53.2
2329	3349	19715	19735	52.5	47.6	5849	19925	19905	51.4	52.4	1.1	211	73.8	41.7	41	52.2
2330	3350	4645	4665	50.2	42.9	5850	4836	4817	51.2	45	0.9	192	75	45.3	41	52.6
2331	3351	18081	18099	51.2	52.6	5851	18702	18685	50.2	50	1	622	76.2	42.4	41	53.5
2332	3352	28522	28542	50.2	42.9	5852	29298	29280	51.4	52.6	1.2	777	78.4	47.2	41	55.1
2333	3353	24179	24199	52.7	42.9	5853	24740	24717	52.5	41.7	0.2	562	76	42.2	41	54
2334	3354	19715	19735	52.5	47.6	5854	19909	19885	52.5	40	0	195	73.3	41	41	52.1
2335	3355	1810	1830	51.2	42.9	5855	2103	2082	52	45.5	0.8	294	75.5	43.5	41	53.3
2336	3356	19716	19737	52.2	45.5	5856	19909	19885	52.5	40	0.3	194	73.1	40.7	41	51.9
2337	3357	19719	19739	50.6	42.9	5857	19909	19885	52.5	40	1.9	191	72.9	40.3	41	51.3
2338	3358	12977	12996	50.2	40	5858	13332	13312	50.9	47.6	0.6	356	76.1	44.1	41	53.4
2339	3359	19721	19745	52.3	40	5859	19909	19885	52.5	40	0.2	189	73	40.7	41	51.9
2340	3360	17608	17627	50.2	45	5860	17769	17749	50	42.9	0.2	162	72.4	40.7	41	50.8
2341	3361	17608	17627	50.2	45	5861	18231	18210	52.2	45.5	2	624	75.2	40.1	41	52.8
2342	3362	19794	19813	50	50	5862	20099	20078	50.5	40.9	0.5	306	74.4	40.8	41	52.2
2343	3363	4658	4677	50.5	50	5863	5306	5289	50.8	50	0.3	649	75.5	40.7	41	53.1
2344	3364	24179	24200	53.3	40.9	5864	24580	24560	51.3	52.4	2	402	74.6	40	41	52.7
2345	3365	19794	19813	50	50	5865	19925	19906	50.1	50	0.1	132	72.8	43.9	41	51.1
2346	3366	1046	1064	51.2	47.4	5866	1498	1481	51	50	0.2	453	76.4	43.9	41	53.9
2347	3367	1046	1064	51.2	47.4	5867	1497	1480	50.3	50	0.9	452	76.5	44	41	53.7
2348	3368	2133	2152	50.7	45	5868	2675	2656	50.4	50	0.3	543	76.8	44.2	41	54
2349	3369	28965	28984	52.9	55	5869	29298	29279	52.6	55	0.3	334	76.4	45.2	41	54.4
2350	3370	24378	24397	55	55	5870	24564	24542	55	47.8	0	187	73.6	42.2	41	53.1
2351	3371	25348	25366	51.2	47.4	5871	25651	25632	52.7	50	1.6	304	74.7	41.4	41	52.7
2352	3372	19794	19813	50	50	5872	19923	19903	50.9	47.6	0.9	130	72.7	43.8	41	51
2353	3373	28967	28987	51.6	52.4	5873	29358	29339	52.8	50	1.2	392	76.5	44.6	41	54.1
2354	3374	17608	17627	50.2	45	5874	18233	18215	51.3	52.6	1.1	626	75.3	40.3	41	52.9
2355	3375	29186	29206	51.3	42.9	5875	29358	29339	52.8	50	1.5	173	74.5	45.1	41	52.6
2356	3376	24379	24398	55	55	5876	25093	25074	54.6	55	0.4	715	75.9	41.4	41	54.6
2357	3377	3170	3191	50.9	45.5	5877	3646	3625	52	40.9	1.1	477	75.7	41.9	41	53.4
2358	3378	3170	3191	50.9	45.5	5878	3647	3628	50.6	45	0.3	478	75.7	42.1	41	53.3
2359	3379	9140	9159	50.1	45	5879	9249	9230	51.5	45	1.4	110	71.3	42.7	41	50
2360	3380	12976	12995	51.1	45	5880	13326	13306	50.7	42.9	0.4	351	76.1	44.2	41	53.6
2361	3381	12976	12995	51.1	45	5881	13328	13307	51.2	45.5	0.1	353	76.1	44.2	41	53.7
2362	3382	3168	3189	51	45.5	5882	3494	3473	50.4	40.9	0.5	327	75.1	42.2	41	52.8
2363	3383	19794	19813	50	50	5883	19917	19896	50.9	45.5	0.9	124	72.3	43.5	41	50.7

2364	3384	24379	24398	55	55	5884	24517	24494	53.2	41.7	1.8	139	72.7	43.2	41	52
2365	3385	24380	24399	55	55	5885	25093	25074	54.6	55	0.4	714	76	41.5	41	54.6
2366	3386	2823	2844	50.4	45.5	5886	3201	3183	50.6	52.6	0.2	379	75.8	43	41	53.3
2367	3387	3798	3819	54.2	50	5887	4318	4294	54.4	40	0.2	521	75.4	41.1	41	54.2
2368	3388	9139	9159	52.5	47.6	5888	9852	9829	53.1	45.8	0.6	714	75.4	40.1	41	53.6
2369	3389	9139	9159	52.5	47.6	5889	9852	9828	53.6	44	1.1	714	75.4	40.1	41	53.6
2370	3390	19795	19814	50.4	45	5890	19927	19908	52.1	55	1.7	133	72.7	43.6	41	51.1
2371	3391	19795	19814	50.4	45	5891	19924	19905	50.1	50	0.3	130	72.4	43.1	41	50.8
2372	3392	12976	12995	51.1	45	5892	13329	13308	50.5	40.9	0.6	354	76.1	44.1	41	53.5
2373	3393	2133	2152	50.7	45	5893	2672	2654	50.9	52.6	0.2	540	76.8	44.3	41	54.1
2374	3394	4593	4613	51.5	47.6	5894	4836	4817	51.2	45	0.3	244	75.3	44.3	41	53.2
2375	3395	1810	1830	51.2	42.9	5895	2113	2094	50.1	45	1.1	304	75.5	43.4	41	53
2376	3396	17036	17058	53.5	47.8	5896	17483	17465	54.4	52.6	0.9	448	75.3	41.3	41	53.9
2377	3397	1046	1064	51.2	47.4	5897	1481	1463	50.5	47.4	0.6	436	76.2	43.6	41	53.6
2378	3398	9055	9079	52.8	40	5898	9255	9236	51.1	45	1.8	201	73.5	41.3	41	51.9
2379	3399	12976	12995	51.1	45	5899	13332	13312	50.9	47.6	0.2	357	76.2	44.3	41	53.7
2380	3400	8865	8884	50.4	45	5900	9311	9292	50.7	50	0.3	447	75.4	41.4	41	53
2381	3401	25363	25381	51.1	52.6	5901	25651	25632	52.7	50	1.6	289	74.3	40.8	41	52.5
2382	3402	3168	3189	51	45.5	5902	3504	3485	50.4	45	0.6	337	75.3	42.4	41	52.9
2383	3403	25363	25381	51.1	52.6	5903	25649	25629	51.5	42.9	0.3	287	74.1	40.4	41	52.3
2384	3404	29182	29202	51.2	42.9	5904	29414	29395	50.5	50	0.7	233	75.5	45.1	41	53.1
2385	3405	3031	3051	51.3	52.4	5905	3497	3478	51.3	50	0.1	467	76.3	43.5	41	53.9
2386	3406	29172	29192	51.5	42.9	5906	29412	29393	50.3	45	1.1	241	75.6	45.2	41	53.2
2387	3407	12040	12057	50.6	50	5907	12412	12392	50	42.9	0.6	373	75.9	43.4	41	53.2
2388	3408	11543	11562	50.4	40	5908	12110	12090	51.1	42.9	0.7	568	75.9	41.9	41	53.4
2389	3409	16909	16928	50.8	45	5909	17038	17021	50.7	50	0.1	130	72.7	43.8	41	51.2
2390	3410	16909	16928	50.8	45	5910	17039	17022	51.4	50	0.6	131	72.6	43.5	41	51.2
2391	3411	18077	18097	51.5	47.6	5911	18233	18214	52	50	0.5	157	73.9	44.6	41	52.3
2392	3412	18077	18097	51.5	47.6	5912	18233	18215	51.3	52.6	0.2	157	73.9	44.6	41	52.2
2393	3413	9055	9079	52.8	40	5913	9252	9234	51.4	52.6	1.4	198	73.7	41.9	41	52.1
2394	3414	25676	25697	51.9	40.9	5914	25832	25810	53.6	47.8	1.7	157	72.1	40.1	41	51.1
2395	3415	2223	2244	51.4	45.5	5915	2676	2657	50.7	50	0.7	454	76.9	45.2	41	54.2
2396	3416	619	640	50.4	45.5	5916	1171	1153	50.4	47.4	0	553	77.9	46.8	41	54.7
2397	3417	11541	11561	50.9	42.9	5917	12110	12090	51.1	42.9	0.3	570	75.9	41.9	41	53.5
2398	3418	3360	3379	50.7	45	5918	3497	3478	51.3	50	0.6	138	74	46.4	42	52.1
2399	3419	19725	19745	50	42.9	5919	19921	19901	50.2	47.6	0.1	197	73.5	41.6	42	51.6
2400	3420	19720	19740	51.3	42.9	5920	19921	19901	50.2	47.6	1.1	202	73.4	41.1	42	51.5
2401	3421	3360	3379	50.7	45	5921	3500	3481	51.2	50	0.5	141	74	46.1	42	52.1
2402	3422	19717	19738	50.8	40.9	5922	19921	19901	50.2	47.6	0.6	205	73.4	41	42	51.5
2403	3423	24562	24580	50.1	52.6	5923	25209	25190	50.6	50	0.5	648	76.1	42	42	53.4
2404	3424	24559	24579	52	52.4	5924	24740	24717	52.5	41.7	0.5	182	76	48.4	42	53.9
2405	3425	3360	3379	50.7	45	5925	3504	3485	50.4	45	0.3	145	74.2	46.2	42	52.2
2406	3426	19716	19737	52.2	45.5	5926	19921	19900	51.8	45.5	0.4	206	73.5	41.3	42	52.1
2407	3427	3232	3251	50.3	50	5927	3494	3473	50.4	40.9	0.1	263	74.3	41.4	42	52.2
2408	3428	26039	26058	54	55	5928	26657	26636	52.6	45.5	1.5	619	75.4	40.5	42	53.7
2409	3429	3232	3251	50.3	50	5929	3504	3485	50.4	45	0.1	273	74.6	41.8	42	52.4
2410	3430	19715	19735	52.5	47.6	5930	19921	19900	51.8	45.5	0.7	207	73.7	41.5	42	52.2
2411	3431	26039	26058	54	55	5931	26653	26631	53.2	43.5	0.9	615	75.3	40.3	42	53.8
2412	3432	3229	3248	50.6	50	5932	3494	3473	50.4	40.9	0.2	266	74.3	41.4	42	52.3
2413	3433	3229	3248	50.6	50	5933	3504	3485	50.4	45	0.3	276	74.5	41.7	42	52.4
2414	3434	3225	3244	52.4	55	5934	3495	3473	51.8	43.5	0.6	271	74.7	42.1	42	52.9

2415	3435	3222	3241	52	50	5935	3650	3631	53.1	50	1.1	429	75.5	42	42	53.6
2416	3436	24559	24579	52	52.4	5936	25209	25190	50.6	50	1.3	651	76.1	42.1	42	53.6
2417	3437	6158	6178	51.3	42.9	5937	6289	6267	52.2	43.5	0.9	132	71.3	40.2	42	50.4
2418	3438	19709	19730	51.3	40.9	5938	19917	19896	50.9	45.5	0.3	209	73.7	41.6	42	52
2419	3439	3223	3241	50.2	52.6	5939	3497	3478	51.3	50	1.1	275	74.7	42.2	42	52.5
2420	3440	3223	3241	50.2	52.6	5940	3646	3625	52	40.9	1.8	424	75.4	41.7	42	53
2421	3441	3223	3241	50.2	52.6	5941	3647	3628	50.6	45	0.4	425	75.5	41.9	42	53
2422	3442	3217	3237	51.8	47.6	5942	3650	3631	53.1	50	1.3	434	75.5	41.9	42	53.5
2423	3443	9352	9372	51.3	42.9	5943	10014	9996	50.7	52.6	0.6	663	75.6	40.7	42	53.2
2424	3444	23733	23752	55.6	55	5944	24022	24003	55.5	55	0.1	290	74.5	41.4	42	53.9
2425	3445	26040	26061	56.4	54.5	5945	26661	26639	55.3	47.8	1.2	622	75.5	40.7	42	54.5
2426	3446	9918	9938	51.4	47.6	5946	10608	10589	51	50	0.4	691	75.8	41.1	42	53.4
2427	3447	7724	7742	51.4	52.6	5947	7843	7825	52.8	52.6	1.3	120	70.7	40	42	50
2428	3448	26040	26061	56.4	54.5	5948	26655	26631	56.2	48	0.2	616	75.4	40.6	42	54.8
2429	3449	28117	28135	50.6	52.6	5949	28506	28488	50.2	47.4	0.4	390	79.4	51.8	42	55.8
2430	3450	3217	3236	51.1	50	5950	3497	3478	51.3	50	0.2	281	74.7	42	42	52.7
2431	3451	3217	3236	51.1	50	5951	3500	3481	51.2	50	0.1	284	74.7	41.9	42	52.7
2432	3452	3165	3187	51.6	43.5	5952	3650	3631	53.1	50	1.5	486	75.8	42.2	42	53.6
2433	3453	19709	19730	51.3	40.9	5953	19925	19906	50.1	50	1.2	217	74	41.9	42	51.9
2434	3454	9927	9945	50.8	52.6	5954	10199	10180	51.5	45	0.7	273	75.3	43.6	42	53.1
2435	3455	9929	9946	50	50	5955	10670	10649	51.3	40.9	1.3	742	75.7	40.8	42	53.1
2436	3456	19709	19730	51.3	40.9	5956	19927	19908	52.1	55	0.9	219	74	42	42	52.3
2437	3457	9934	9953	50.7	50	5957	10356	10336	52.4	47.6	1.7	423	75.6	42.1	42	53.2
2438	3458	19709	19730	51.3	40.9	5958	19930	19910	50.6	47.6	0.7	222	74	41.9	42	52.1
2439	3459	3164	3186	51.6	43.5	5959	3650	3631	53.1	50	1.5	487	75.9	42.3	42	53.7
2440	3460	3089	3110	51.8	45.5	5960	3188	3166	51.6	43.5	0.2	100	72	46	42	51
2441	3461	18979	19000	51.6	45.5	5961	19215	19194	50.2	40.9	1.4	237	73.5	40.1	42	51.6
2442	3462	26421	26441	51.5	42.9	5962	26900	26882	51.5	52.6	0.1	480	77.5	46.2	42	54.8
2443	3463	26421	26441	51.5	42.9	5963	26828	26810	52.9	52.6	1.4	408	76.6	44.9	42	54.2
2444	3464	11540	11557	50.4	50	5964	11826	11802	51.3	40	0.8	287	74.4	41.1	42	52.3
2445	3465	26421	26441	51.5	42.9	5965	26695	26678	50.5	50	1	275	74.9	42.5	42	52.7
2446	3466	11540	11557	50.4	50	5966	11819	11798	50.3	40.9	0.1	280	74.3	41.1	42	52.2
2447	3467	11540	11557	50.4	50	5967	11817	11797	50.4	42.9	0.1	278	74.3	41	42	52.2
2448	3468	23841	23859	50.5	52.6	5968	24515	24494	50.4	40.9	0.1	675	76.1	41.9	42	53.5
2449	3469	3055	3077	52.8	43.5	5969	3495	3473	51.8	43.5	0.9	441	76	43.1	42	53.9
2450	3470	3795	3813	52.1	52.6	5970	4565	4542	53.9	41.7	1.8	771	75.6	40.3	42	53.6
2451	3471	11540	11560	53.2	47.6	5971	11984	11966	53	52.6	0.2	445	75.1	40.7	42	53.6
2452	3472	11541	11561	50.9	42.9	5972	12165	12147	51.2	47.4	0.4	625	75.7	41.3	42	53.4
2453	3473	3795	3815	54.6	52.4	5973	4318	4294	54.4	40	0.2	524	75.5	41.2	42	54.3
2454	3474	7723	7741	52.2	52.6	5974	7853	7833	50.7	47.6	1.5	131	71.3	40.5	42	50.2
2455	3475	3055	3075	51.8	47.6	5975	3504	3485	50.4	45	1.4	450	76.1	43.1	42	53.5
2456	3476	3055	3074	51.1	50	5976	3494	3473	50.4	40.9	0.7	440	76	43	42	53.4
2457	3477	26421	26441	51.5	42.9	5977	26651	26631	50.2	42.9	1.3	231	73.8	41.1	42	51.8
2458	3478	28109	28130	50.2	40.9	5978	28672	28654	50.6	52.6	0.4	564	79.9	51.6	42	56.1
2459	3479	3055	3074	51.1	50	5979	3504	3485	50.4	45	0.7	450	76.1	43.1	42	53.5
2460	3480	12232	12250	51.9	52.6	5980	12412	12392	50	42.9	1.9	181	73.2	41.4	42	51.3
2461	3481	3034	3053	50.3	50	5981	3210	3190	50.5	47.6	0.2	177	74.9	45.8	42	52.6
2462	3482	3034	3053	50.3	50	5982	3494	3473	50.4	40.9	0.1	461	76.1	43.2	42	53.5
2463	3483	3034	3053	50.3	50	5983	3504	3485	50.4	45	0.1	471	76.2	43.3	42	53.5
2464	3484	12236	12256	51.2	42.9	5984	12498	12480	50	47.4	1.1	263	74.6	42.2	42	52.4
2465	3485	12352	12375	52.9	41.7	5985	12724	12705	52.4	55	0.5	373	75.7	42.9	42	53.8

2466	3486	26421	26441	51.5	42.9	5986	26585	26567	51	47.4	0.5	165	72.2	40	42	50.9
2467	3487	3031	3051	51.3	52.4	5987	3503	3484	51.5	50	0.1	473	76.3	43.6	42	53.9
2468	3488	18704	18724	50.8	47.6	5988	19480	19459	50.3	40.9	0.4	777	75.5	40.2	42	53.1
2469	3489	3016	3036	50.2	42.9	5989	3646	3625	52	40.9	1.8	631	76.4	42.8	42	53.6
2470	3490	2823	2844	50.4	45.5	5990	3053	3034	50.3	50	0.2	231	74	41.6	42	52
2471	3491	12366	12384	51.7	52.6	5991	12994	12976	50.3	47.4	1.3	629	76.4	42.9	42	53.7
2472	3492	12366	12384	51.7	52.6	5992	12992	12974	51.2	52.6	0.5	627	76.5	43.1	42	54
2473	3493	2823	2844	50.4	45.5	5993	3056	3037	52.1	55	1.6	234	74.2	41.9	42	52.2
2474	3494	2522	2541	51.4	45	5994	2672	2654	50.9	52.6	0.5	151	75.3	48.3	42	53
2475	3495	2522	2541	51.4	45	5995	2675	2656	50.4	50	1	154	75.2	48.1	42	52.9
2476	3496	2429	2447	50.2	47.4	5996	3056	3037	52.1	55	1.9	628	76.3	42.7	42	53.6
2477	3497	2429	2447	50.2	47.4	5997	3190	3169	50.7	45.5	0.5	762	76.6	42.9	42	53.8
2478	3498	27436	27455	52.7	45	5998	27541	27521	51.7	47.6	1	106	72.1	45.3	42	51.1
2479	3499	2429	2447	50.2	47.4	5999	3192	3171	51.9	50	1.7	764	76.7	43.1	42	53.8
2480	3500	2427	2445	52.1	52.6	6000	3056	3037	52.1	55	0	630	76.4	42.9	42	54.2
2481	3501	27389	27407	50.6	47.4	6001	27541	27521	51.7	47.6	1.1	153	73.2	43.1	42	51.5
2482	3502	2427	2445	52.1	52.6	6002	3190	3169	50.7	45.5	1.4	764	76.7	43.1	42	54
2483	3503	18616	18636	51.4	47.6	6003	19316	19295	50	40.9	1.4	701	75.4	40.2	42	52.9
2484	3504	2377	2395	52.4	52.6	6004	2672	2653	51.6	50	0.8	296	77	47.3	42	54.5
2485	3505	18591	18611	51.7	42.9	6005	19216	19195	50.2	40.9	1.4	626	75.7	41.1	42	53.1
2486	3506	12366	12384	51.7	52.6	6006	12739	12718	51	40.9	0.7	374	75.6	42.8	42	53.3
2487	3507	2377	2395	52.4	52.6	6007	2672	2654	50.9	52.6	1.5	296	77	47.3	42	54.3
2488	3508	16982	17001	51.2	55	6008	17111	17090	51.1	40.9	0.1	130	74.6	48.5	42	52.6
2489	3509	2377	2395	52.4	52.6	6009	2675	2656	50.4	50	2	299	77	47.2	42	54.1
2490	3510	18590	18608	50.6	42.1	6010	19216	19195	50.2	40.9	0.3	627	75.6	41	42	53.1
2491	3511	2377	2395	52.4	52.6	6011	2891	2873	50.8	47.4	1.6	515	76.8	44.5	42	54.1
2492	3512	8220	8240	54	47.6	6012	8935	8917	54.5	52.6	0.4	716	75.4	40.1	42	54.1
2493	3513	12370	12388	50.1	47.4	6013	12998	12979	50.1	45	0.1	629	76.4	42.9	42	53.6
2494	3514	2223	2244	51.4	45.5	6014	2675	2656	50.4	50	1	453	77	45.3	42	54.1
2495	3515	2220	2239	51.3	45	6015	2672	2654	50.9	52.6	0.4	453	77	45.3	42	54.2
2496	3516	24418	24439	52.9	45.5	6016	24936	24919	51.8	50	1.1	519	76	42.4	42	53.8
2497	3517	18586	18603	50.4	44.4	6017	19216	19195	50.2	40.9	0.2	631	75.6	40.9	42	53.1
2498	3518	2220	2239	51.3	45	6018	2675	2656	50.4	50	0.8	456	76.9	45.2	42	54.1
2499	3519	1402	1422	50.2	42.9	6019	2153	2134	50.4	45	0.2	752	76.7	43.1	42	53.8
2500	3520	1356	1375	53.8	55	6020	2153	2133	52.1	42.9	1.7	798	76.9	43.5	42	54.5

Table 5: Primers

Forward primer SEQ ID NO & Co-ordinates		Reverse primer SEQ ID NO & Co-ordinates		T_M (FOR & REV) (°C)		Product length (bp)
6076	1-19	6171	199-183	50.1	50.3	199
6077	149-169	6172	334-315	51.5	52.4	186
6078	292-310	6173	560-541	50.8	51.1	269
6079	598-619	6174	749-731	52.6	50.6	152
6080	721-742	6175	930-912	50.4	50.3	210
6081	888-912	6176	1077-1058	52.8	51.2	190
6082	984-1003	6177	1149-1131	51.1	51.1	166

6083	1157-1175	6178	1479-1460	50.9	51.6	323
6084	1420-1441	6179	1700-1680	51.2	50.7	281
6085	1685-1707	6180	1834-1811	53.8	53.7	150
6086	1740-1764	6181	1987-1963	53.4	52.2	248
6087	2007-2025	6182	2251-2232	50.3	50.1	245
6088	2226-2245	6183	2385-2366	50.4	50.1	160
6089	2428-2446	6184	2749-2728	50.1	50.3	322
6090	2742-2763	6185	2893-2875	50.6	51.4	152
6091	2823-2844	6186	3082-3058	50.4	52.3	260
6092	3007-3031	6187	3185-3164	51.9	51.0	179
6093	3234-3254	6188	3497-3478	51.1	51.3	264
6094	3453-3476	6189	3647-3627	51.8	52.1	195
6095	3601-3622	6190	3877-3853	52.5	53.6	277
6096	4007-4027	6191	4158-4135	51.1	51.4	152
6097	4141-4165	6192	4316-4295	51.3	50.8	176
6098	4366-4387	6193	4567-4544	54.6	55.4	202
6099	4488-4508	6194	4708-4690	50.7	50.3	221
6100	4658-4677	6195	4994-4974	50.5	51.2	337
6101	4902-4922	6196	5115-5092	50.5	51.4	214
6102	5239-5260	6197	5450-5430	50.8	50.9	212
6103	5366-5389	6198	5560-5542	50.5	51.8	195
6104	5593-5612	6199	5860-5836	50.8	51.6	268
6105	6042-6062	6200	6291-6271	50.4	51.1	250
6106	6271-6291	6201	6483-6463	51.1	50.2	213
6107	7017-7040	6202	7171-7153	52.4	52.8	155
6108	7253-7272	6203	7504-7486	50.3	50.3	252
6109	7415-7434	6204	7677-7654	54.5	53.6	263
6110	7615-7635	6205	7821-7798	51.1	52.8	207
6111	7728-7746	6206	7936-7915	51.7	50.1	209
6112	7845-7867	6207	7994-7970	52.7	53.4	150
6113	8011-8029	6208	8189-8170	51.4	50.6	179
6114	8143-8166	6209	8300-8281	52.2	50.8	158
6115	8221-8239	6210	8388-8369	51.0	51.1	168
6116	8553-8575	6211	8931-8915	51.8	50.3	379
6117	8867-8886	6212	9254-9236	50.7	50.6	388

6118	9244-9267	6213	9597-9573	51.9	53.4	354
6119	9620-9640	6214	9990-9969	51.3	51.3	371
6120	10009-10027	6215	10188-10171	50.2	50.2	180
6121	10093-10113	6216	10244-10223	52.4	50.6	152
6122	10242-10265	6217	10608-10589	51.2	51.0	367
6123	10549-10571	6218	10783-10763	53.7	55.2	235
6124	10766-10785	6219	10930-10912	52.0	51.1	165
6125	11065-11085	6220	11305-11287	50.7	50.0	241
6126	11265-11287	6221	11429-11405	54.5	53.5	165
6127	11552-11571	6222	11730-11709	52.0	50.4	179
6128	11705-11726	6223	11869-11848	50.1	50.2	165
6129	11801-11824	6224	11984-11967	51.5	50.4	184
6130	12040-12058	6225	12254-12235	52.3	51.9	215
6131	12235-12253	6226	12406-12388	50.1	50.1	172
6132	12366-12384	6227	12730-12712	51.7	52.2	365
6133	12727-12748	6228	12994-12976	50.8	50.3	268
6134	12948-12966	6229	13224-13201	50.7	51.7	277
6135	13175-13196	6230	13324-13300	54.3	55.1	150
6136	13237-13258	6231	13545-13526	52.9	52.9	309
6137	13790-13810	6232	13963-13945	50.9	50.7	174
6138	14080-14098	6233	14280-14257	51.5	51.0	201
6139	14405-14427	6234	14561-14540	50.2	50.9	157
6140	14882-14906	6235	15046-15024	50.9	51.5	165
6141	14951-14976	6236	15145-15124	53.1	52.9	195
6142	15113-15134	6237	15275-15257	51.6	50.8	163
6143	15211-15230	6238	15383-15363	50.2	50.1	173
6144	15364-15387	6239	15528-15506	54.0	52.1	165
6145	15456-15477	6240	15605-15585	52.0	53.2	150
6146	15513-15532	6241	15897-15876	51.2	50.4	385
6147	15837-15856	6242	15999-15978	52.3	50.8	163
6148	16073-16096	6243	16301-16277	51.7	52.8	229
6149	16245-16266	6244	16404-16380	50.3	52.0	160
6150	16366-16385	6245	16515-16492	52.9	53.8	150
6151	16553-16571	6246	16777-16758	53.4	51.5	225
6152	16832-16852	6247	17026-17004	51.0	51.6	195

6153	16982-17001	6248	17359-17340	51.2	50.2	378
6154	17354-17372	6249	17511-17490	51.3	50.4	158
6155	17422-17443	6250	17573-17552	50.2	51.1	152
6156	17603-17623	6251	17769-17748	50.7	51.5	167
6157	17728-17746	6252	17883-17862	50.9	51.2	156
6158	18011-18030	6253	18163-18140	52.9	51.9	153
6159	18076-18098	6254	18225-18205	54.4	55.0	150
6160	18270-18292	6255	18432-18413	51.9	51.4	163
6161	18352-18373	6256	18648-18629	51.3	50.8	297
6162	18550-18571	6257	18702-18684	50.4	51.9	153
6163	18720-18738	6258	19004-18983	50.6	51.0	285
6164	18960-18981	6259	19109-19085	54.7	54.3	150
6165	19065-19089	6260	19217-19195	52.8	51.7	153
6166	19310-19329	6261	19476-19454	50.2	52.1	167
6167	19569-19589	6262	19719-19701	50.5	51.8	151
6168	19707-19731	6263	19856-19833	55.7	55.9	150
6169	19771-19792	6264	19921-19901	50.1	50.2	151
6170	19833-19851	6265	19986-19966	50.9	50.7	154

Table 6: Primers

Forward primer SEQ ID NO & Co-ordinates		Reverse primer SEQ ID NO & Co-ordinates		T_M (FOR & REV) (°C)		Product length (bp)
6266	20110-20132	6305	20425-20404	51.9	50.9	316
6267	20468-20492	6306	20617-20596	53.2	53.5	150
6268	20557-20578	6307	20891-20871	50.4	50.6	335
6269	20838-20856	6308	21037-21015	52.5	52.0	200
6270	21096-21116	6309	21295-21272	50.1	51.7	200
6271	22173-22194	6310	22414-22395	52.4	51.0	242
6272	22320-22342	6311	22501-22479	54.8	54.3	182
6273	22532-22552	6312	22695-22675	50.6	50.0	164
6274	22712-22736	6313	22873-22852	56.7	55.5	162
6275	22842-22861	6314	23086-23067	51.0	52.8	245
6276	23151-23170	6315	23395-23376	51.4	50.3	245
6277	23307-23326	6316	23524-23501	51.1	51.1	218
6278	23615-23635	6317	23776-23758	50.7	50.2	162

6279	23838-23857	6318	23996-23977	50.4	50.6	159
6280	24030-24051	6319	24407-24386	57.6	55.7	378
6281	24388-24407	6320	24581-24563	50.4	50.1	194
6282	24559-24579	6321	24938-24921	52.0	50.4	380
6283	24922-24941	6322	25184-25166	50.1	51.2	263
6284	25201-25220	6323	25400-25382	51.1	51.4	200
6285	25363-25381	6324	25646-25627	51.1	50.5	284
6286	25656-25681	6325	25839-25814	54.5	56.4	184
6287	25761-25782	6326	25982-25961	54.6	54.3	222
6288	26039-26058	6327	26189-26166	54.0	53.0	151
6289	26184-26205	6328	26333-26310	50.9	51.8	150
6290	26422-26442	6329	26660-26641	51.3	50.2	239
6291	26571-26589	6330	26739-26715	51.7	53.2	169
6292	26733-26752	6331	26960-26941	51.1	52.2	228
6293	26866-26885	6332	27139-27117	50.7	51.9	274
6294	27300-27321	6333	27458-27439	51.2	50.2	159
6295	27361-27380	6334	27579-27558	52.4	51.1	219
6296	27718-27740	6335	27917-27901	50.7	50.0	200
6297	28041-28059	6336	28207-28189	50.8	50.8	167
6298	28166-28189	6337	28411-28393	52.2	52.9	246
6299	28395-28414	6338	28671-28653	51.5	50.2	277
6300	28654-28672	6339	28821-28800	50.6	52.3	168
6301	28867-28885	6340	29184-29166	51.5	51.6	318
6302	29183-29204	6341	29360-29342	50.4	50.4	178
6303	29262-29279	6342	29626-29606	50.1	50.2	365
6304	29538-29559	6343	29690-29670	50.0	50.4	153

Table 7: Primers

Name	SEQ ID NO:	Co-ordinates		Name	SEQ ID NO:	Co-ordinates
AB4f	6344	19869-19888		CB1r	6367	28011-28030
AB5f	6345	20238-20257		CB2r	6368	27671-27690
BC1f	6346	20581-20600		CB3r	6369	27301-27320
BC2f	6347	20950-20969		CB4r	6370	26931-26950
BC3f	6348	21339-21358		CB5r	6371	26575-26594
BC4f	6349	21708-21727		CB6r	6372	26191-26210
BC5f	6350	22041-22060		CB7r	6373	25841-25860
BC6f	6351	22410-22429		CB8r	6374	25476-25495
BC7f	6352	22759-22778		CB9r	6375	25126-25145

BC8f	6353	23131-23150		CB10r	6376	24791-24810
BC9f	6354	23500-23519		CB11r	6377	24422-24441
BC10f	6355	23841-23860		CB12r	6378	24031-24050
BC11f	6356	24210-24229		CB13r	6379	23673-23692
BC12f	6357	24560-24579		CB14r	6380	23298-23317
BC13f	6358	24941-24960		CB15r	6381	22928-22947
BC14f	6359	25310-25329		CB16r	6382	22567-22586
BC15f	6360	25675-25694		CB17r	6383	22196-22215
BC16f	6361	26044-26063		CB18r	6384	21831-21850
BC17f	6362	26413-26432		CB19r	6385	21431-21450
BC18f	6363	26763-26782		CB20r	6386	21073-21092
BC19f	6364	27132-27151		CB21r	6387	20715-20734
BC20f	6365	27491-27510		BA1r	6388	20345-20364
BC21f	6366	27845-27864		BA2r	6389	19969-19988
				BA3r	6390	19599-19618
				BA4r	6391	19228-19247
				BA5r	6392	18852-18871

Table 8: Primers

Name	SEQ ID NO	Co-ordinates	Name	SEQ ID NO	Co-ordinates
F1	6393	1-19	R1	6441	334-315
F2	6394	292-310	R2	6442	749-731
F3	6395	721-742	R3	6443	1077-1058
F4	6396	984-1003	R4	6444	1479-1460
F5	6397	1420-1441	R5	6445	1834-1811
F6	6398	1740-1764	R6	6446	2251-2232
F7	6399	2226-2245	R7	6447	2749-2728
F8	6400	2742-2763	R8	6448	3082-3058
F9	6401	3007-3031	R9	6449	3497-3478
F10	6402	3453-3476	R10	6450	3877-3853
F11	6403	4007-4027	R11	6451	4316-4295
F12	6404	4366-4387	R12	6452	4708-4690
F13	6405	4658-4677	R13	6453	5115-5092
F14	6406	5239-5260	R14	6454	5560-5542
F15	6407	5593-5612	R15	6455	6291-6271
F16	6408	6271-6291	R16	6456	7171-7153
F17	6409	7253-7272	R17	6457	7677-7654
F18	6410	7615-7635	R18	6458	7936-7915
F19	6411	7845-7867	R19	6459	8189-8170
F20	6412	8143-8166	R20	6460	8388-8369
F21	6413	8553-8575	R21	6461	9254-9236
F22	6414	9244-9267	R22	6462	9990-9969
F23	6415	10009-10027	R23	6463	10244-10223
F24	6416	10242-10265	R24	6464	10783-10763
F25	6417	10766-10785	R25	6465	11305-11287
F26	6418	11265-11287	R26	6466	11730-11709
F27	6419	11705-11726	R27	6467	11984-11967
F28	6420	12040-12058	R28	6468	12406-12388
F29	6421	12366-12384	R29	6469	12994-12976
F30	6422	12948-12966	R30	6470	13324-13300
F31	6423	13237-13258	R31	6471	13963-13945

F32	6424	14080-14098	R32	6472	14561-14540
F33	6425	14882-14906	R33	6473	15145-15124
F34	6426	15113-15134	R34	6474	15383-15363
F35	6427	15364-15387	R35	6475	15605-15585
F36	6428	15513-15532	R36	6476	15999-15978
F37	6429	16073-16096	R37	6477	16404-16380
F38	6430	16366-16385	R38	6478	16777-16758
F39	6431	16832-16852	R39	6479	17359-17340
F40	6432	17354-17372	R40	6480	17573-17552
F41	6433	17603-17623	R41	6481	17883-17862
F42	6434	18011-18030	R42	6482	18225-18205
F43	6435	18270-18292	R43	6483	18648-18629
F44	6436	18550-18571	R44	6484	19004-18983
F45	6437	18960-18981	R45	6485	19217-19195
F46	6438	19310-19329	R46	6486	19719-19701
F47	6439	19707-19731	R47	6487	19921-19901
F48	6440	19833-19851			

Table 9: Primers

	Name	SEQ ID NO:
1	CB12R	6488
2	R0010	6489
3	R0011	6490
4	R0012	6491
5	BNI-ED	6492
6	BNI-EU	6493
7	SAR1S-U	6494
8	SAR1As-D	6495
9	SAR1S	6496
10	SAR1As	6497
11	IN2-U	6498
12	IN4-D	6499
13	IN-2	6500
14	IN-4	6501
15	IN-6	6502
16	IN-7	6503
17	COR1-U	6504
18	COR2-D	6505
19	COR-1	6506
20	COR-2	6507
21	HKUF-U	6508
22	HKUR-D	6509
23	HKU-F	6510
24	HKU-R	6511
25	1451-D	6512
26	1451-U	6513
27	690-D	6514
28	690-U	6515
29	690-D2	6516

	Name	SEQ ID NO:
37	EMC8-D2	6524
38	EMC8-U2	6525
39	EMC11-D	6526
40	EMC11-U	6527
41	ORF1B-D	6528
42	ORF1B-U	6529
43	ORFS-D	6530
44	ORFS-U	6531
45	E7-717F	6532
46	E8-85R	6533
47	E8-307F	6534
48	E11-771F	6535
49	E11-96R	6536
50	CON1-F	6537
51	CON1-U	6538
52	CON2-F	6539
53	CON2-R	6540
54	CON3-F	6541
55	CON3-R	6542
56	15-F	6543
57	15-R	6544
58	15-F2	6545
59	15-R2	6546
60	13-F	6547
61	13-R	6548
62	13-F2	6549
63	13-R2	6550
64	CONTIG-F	6551
65	QT3-R	6552

30	690-U2	6517
31	EMC7-D	6518
32	EMC7-U	6519
33	EMC7-D2	6520
34	EMC7-U2	6521
35	EMC8-D	6522
36	EMC8-U	6523

66	QT3-F	6553
67	QIN-R	6554
68	QIN-F	6555
69	AB1-F	6556
70	AB2-F	6557
71	AB3-F	6558
72	AB1-R	6559

Table 10: Features of the predicted proteins and open reading frames of the SARS virus

	SARS ORF (SEQ ID NO)	Length (aa)	Role	Cleavage site	Features	Cons[d] *
ORF1a	P28 (9766)	179	Leader protein	179 (G/G)[#]		+
	P65 (9767)	639	Homologue of MHV p65 cleavage product	818 (G/A)		+
	Nsp1 (9768)	2422 [##]	Papain like protease, cleaves the first two proteins	3240 (Q/S)	phosphoesterase domain Zn binding domain	+
	Nsp2 (9769)	306	3C-like protease, cleaves proteins nsp1-nsp12	3546 (Q/G)		+
	Nsp3 (9770)	290	?	3836 (Q/S)	5 TMDs	+
	Nsp4 (9771)	83	?	3919 (Q/A)	1 TMD	+
	Nsp5 (9772)	198	?	4117 (Q/N)		+
	Nsp6 (9773)	113	?	4230 (Q/A)		+
	Nsp7 (9774)	139	?	4369 (Q/S)	Putative growth factor-like motif	+
ORF1b	Nsp9 (9775)	932	RNA polymerase	5298 (Q/A)		+
	Nsp10 (9776)	601	Putative helicase Tanner et al. (2003) J Biol Chem 278:39578-82	5899 (Q/A)	Metal binding domain, ATP/GTP binding domain	+
	Nsp11 (9777)	527	?	6426 (Q/S)		+
	Nsp12 (9778)	346	?	6772 (Q/A)		+
	Nsp13 (9779)	298	?	-		+
Structural region	Spike (S) (6042)		Major antigenic determinant, contains the receptor-binding domain		Leader peptide, 1 TMD, 17 N-glycosylation sites	+
	Orf3 (6043)	274	?		2 TMDs, 1 N-glycosylation site, 10 O-glycosylation sites	-
	Orf4 (6044)	154	?			-
	Envelope (E) (6045)	76	Associated with viral envelope		1 TMD, 2 N-glycosylation sites	+
	Matrix (M) (6046)	221	Associated with viral envelope, membrane spanning protein		3 TMDs, 1 N-glycosylation site	+
	Orf7 (6047)	63	?		1 TMD	-
	Orf8 (6048)	122	?		1 TMD	-
	Orf9 (6049)	44	?		Surface-associated	-
	Orf10	39	?		Surface-associated	-
	Orf11(6050)	84	?		1 N-glycosylation site	-
	Nucleocapsid (N) (6052)	422	Associated with viral genomic RNA		phosphoprotein	+
	Orf13	98	?		1 O-glycosylation site	-

TMD: predicted transmembrane domain.
Cons[d] *: + indicates presence of corresponding protein at least in one of the other coronaviruses
#: Alternatively, cleaved after Gly-Gly (*i.e.* at G/A) to give a 180mer
##: This 2422mer may be further cleaved after residue 1922 (Gly-2740 of SEQ ID NO: 6039) to give a 1922mer PLpro containing the Zn-binding motif (SEQ ID NO: 7254) and a 500mer.

Table 11: Protein homologies between SARS and other coronaviruses

Numbers indicate percentage of aminoacid identity between SARS proteins and corresponding gene products of other coronaviruses. More conserved pairs are in bold; more variable pairs are underlined.

	group 1			group 2		group 3
Proteins	229E	TGV	PEDV	MHV	BCoV	AIBV
REPLICASE REGION						
leader protein p28	<20	<20	<20	**27**	<20	<20
p65 homologue	<20	**23**	23	<20	20	<20
nsp1 (PLP protease)	25.5	25.8	25.4	29	**30**	25
nsp2 (3CL protease)	40.4	43.8	44.6	**50**	48.4	41
nsp3	30	27	29.4	34.2	**35.5**	28.5
nsp4	38.6	42.2	39.8	**47.5**	46.1	37.3
nsp5	**48.2**	42.9	43.9	46.8	47.3	38.7
nsp6	45.1	38.9	45.1	45.1	**46.9**	39.8
nsp7	53.8	54.5	56.1	56.2	55.4	**58.3**
nsp9 (polymerase)	59.8	59.6	60	**67.3**	66.9	62.4
nsp10 (helicase)	60.7	62	62.3	67.2	68.6	58.9
nsp11	52.3	53.7	52.3	**57.6**	57.6	52
nsp12	43.1	43	45.4	**45.9**	45	40.2
nsp13	56.4	54.4	55.3	63	**65**	53.4
STRUCTURAL REGION						
Spike (S)	28.8	31.6*	30.3	**31.1**	31	32.7*
Envelope (E)	33*	**27.9**	20	23	26.5	23.2
Matrix glycoprotein (M)	30.6	32.5	34.8	40.8	**41.9**	32.5
Nucleocapsid (N)	26.9	30.1	29.5	37.3	**37.4**	31.5

* These three alignments were obtained only on a fragment of the whole protein.

Table 12: Nucleotide and aminoacid differences between five SARS isolates

		FRA*	TOR2*	Urbani*	CUHK*	HKU*
	position°	base/aminoacid	base/aminoacid	base/aminoacid	base/aminoacid	base/aminoacid
ORF1a	2557	A/Thr	G/Ala	G/Ala	G/Ala	G/Ala
	2601	T/Val				C
	7746	G/Pro			T	
	7919	C/Ala		T/Val		
	7930	G/Asp				A/Asn
	8387	G/Ser				C/Thr
	8416	G/Arg				C/Thr
	9404	T/Val			C/Ala	
	9479	T/Val			C/Ala	
	11448	T/Ile	C	C	C	C
ORF1b	13494	GT/Val				AG/Ser
	16622	C/Ala		T		
	17564	T/Asp			C/Glu	
	17846	C/Arg			T	
	18065	G/Lys				A
	18965	A/Ile	T	T	T	T
	19064	A/Glu		G	G	
	19084	T/Ile	C/Thr	C/Thr	C/Thr	C/Thr
spike	21721	G/Gly			A/Asp	
	22222	T/Ile			C/Thr	
	23220	T/Ser	G/Ala			
	24872	T/Leu		C		
	24933	T/Phe	C/Leu	C/Leu	C/Leu	C/Leu
ORF3	25298	G/Gly	A/Arg			
	25569	T/Met				A/Lys
matrix	26600	T/Val	C/Ala	C/Ala	C/Ala	
	26857	T/Ser		C/Pro		
ORF10	27827	T/Cys			C/Arg	
nucleocapsid	28268	T/Ile	C/Thr	C/Thr	C/Thr	C/Thr

* SARS coronavirus FRA (accession number AY310120)
SARS coronavirus TOR2 (accession number AY274119)
SARS coronavirus Urbani (accession number AY278741)
SARS coronavirus CUHK-W1 (accession number AY278554)
SARS coronavirus HKU-39849 (accession number AY278491)

° The position is based on the FRA sequence.

TABLES 13-25: T-epitope predictions for SEQ ID NOS: 6039-6050 & 6052

Epitope predictions were performed at *http://www.mpiib-berlin.mpg.de/MAPPP/binding.html* using a minimum score of 0.5 and the BIMAS matrix, with a maximum of 20 results being selected. The analysis revealed 9mer and 10mer epitopes.

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Table 13: Epitopes for SEQ ID NO: 6039

HLA A1 - 9 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	1867	SEQ ID NO: 7400	8 %	450
2	4139	SEQ ID NO: 7401	5.55 %	312.5
3	88	SEQ ID NO: 7402	4 %	225
4	4249	SEQ ID NO: 7403	3.55 %	200
5	4059	SEQ ID NO: 7404	2.22 %	125
6	2027	SEQ ID NO: 7405	1.6 %	90
7	3413	SEQ ID NO: 7406	1.11 %	62.5
8	1823	SEQ ID NO: 7407	0.88 %	50
9	2798	SEQ ID NO: 7408	0.88 %	50
10	220	SEQ ID NO: 7409	0.8 %	45
11	3738	SEQ ID NO: 7410	0.8 %	45
12	4182	SEQ ID NO: 7411	0.8 %	45
13	4174	SEQ ID NO: 7412	0.66 %	37.5
14	1940	SEQ ID NO: 7413	0.55 %	31.25
15	38	SEQ ID NO: 7414	0.48 %	27
16	1231	SEQ ID NO: 7415	0.44 %	25
17	1613	SEQ ID NO: 7416	0.44 %	25
18	3645	SEQ ID NO: 7417	0.44 %	25
19	4192	SEQ ID NO: 7418	0.44 %	25
20	378	SEQ ID NO: 7419	0.4 %	22.5

HLA A1 - 10 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	1867	SEQ ID NO: 7420	8 %	450
2	1495	SEQ ID NO: 7421	4 %	225
3	3921	SEQ ID NO: 7422	2.4 %	135
4	486	SEQ ID NO: 7423	2.22 %	125
5	4139	SEQ ID NO: 7424	2.22 %	125